Cyclin-Dependent Kinase Inhibition by Flavoalkaloids

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Abstract: Chromone alkaloids and flavoalkaloids are an important group of natural products possessing promising medicinal properties. A chromone alkaloid rohitukine is a major bioactive chemical constituent of plant *Dysoxylum binectariferum* (Meliaceae) Hook. which is phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd. used for treatment of rheumatoid arthritis. This chromone alkaloid led to discovery of two synthetic flavoalkaloids: flavopiridol (Sanofi) and P-276-00 (Piramal) which have reached to advanced stages of clinical development for cancer treatment. Flavopiridol (Alvocidib; L868275; HMR-1275; NSC 649890 of Sanofi-Aventis + NCI) is approved as an orphan drug for treatment of chronic lymphocytic leukemia and is currently undergoing phase II studies as monotherapy and also as in combination regimes with traditional chemotherapy agents. P-276-00 (**12**) is currently in phase II clinical studies for advanced refractory neoplasms and multiple myeloma. Extensive amount of medicinal chemistry efforts have been reported on these flavoalkaloids. Flavopiridol demonstrated potent and specific *in vitro* inhibition of variety of cyclin-dependent kinases with clear block in cell cycle progression at the G1/S and G2/M phases. Preclinical studies demonstrated the capacity of flavopiridol to induce programmed cell death, promote differentiation, inhibit angiogenic processes and modulate transcriptional events. The co-crystallised structure of deschloro-flavopiridol with CDK-2 is available and key interactions in the ATP binding site have been reported. Flavopiridol has also been studied for the treatment of arthritis and atherosclerotic plaque formation. The present review comprises discovery, medicinal chemistry, pharmacology and preclinical/clinical development of flavoalkaloids as CDK inhibitors.

Keywords: Cancer, cyclin-dependent kinase, *Dysoxylum binectariferum,* flavoalkaloid, flavopiridol, P-276-00, rohitukine.

1. INTRODUCTION

Flavones are natural products of the benzopyran class, constituting an important group of flavonoids that are widely distributed in the plant kingdom as secondary metabolites [1]. Large number of flavones have been isolated from natural sources and many of these molecules possess medicinal properties as well as wide array of activities in plants. They protect plants from UV radiation [2], attract insects for pollination [3] and participate in interactions with soil microbes [4]. Flavones with various substitution patterns have been isolated from different parts of plants. The diversity of structure gives them wide range of biological activities against both metabolic and infective diseases. Several reviews have described biological activity profile of different flavones [5-13]. Flavonoids are present in fruits and vegetables and constitute important part of diet and exert beneficial effects on human health [14]. They possess free radical-scavenging and metal ion-chelating abilities [15-17]. In addition, flavonoids, either natural or synthetic, are known to possess antitumor properties [8, 9, 12, 18, 19] through the inhibition of aromatase [20, 21] or topoisomerases [22]. They are also known for their ability to inhibit important cell signaling enzymes such as protein kinase C [23, 24], cyclin-dependent kinases [18, 25, 26] and tyrosine kinases [10, 27].

Cyclin-dependent kinases (CDKs) have been recognized as key regulators of cell cycle progression. Alteration and deregulation of CDK activity have pathogenic link to the cancer. A number of cancers are associated with hyper-activation of CDKs as a result of mutation of the CDK genes or CDK inhibitor genes. Therefore, CDK inhibitors or modulators are of great interest to explore as novel therapeutic agents against cancer [28]. The ATP-competitive CDK inhibitors can be divided into two different classes according to their selectivity. The first class describes the unspecific kinase inhibitors, which can block CDKs and other unrelated serine/threonine and tyrosine kinases in similar concentrations such as staurosporine and its analogue UCN-01. The second class covers selective CDK inhibitors which block all subgroups of this family in an equipotent manner (for example, flavopiridol) and those that show a strong preference for one of these groups. Because CDK inhibitors are ATP competitive ligands; hence earlier they were typically described as purine class of compounds for example dimethylaminopurine, a first substance to be known as a CDK inhibitor [29], olomoucine [30] and roscovitine [31]. The IC_{50} values of these purine class of compounds for CDK1/cyclin B are 120, 7 and 0.2-0.8 μM respectively [32]. Some of the more potent members of this series have been prepared by the Schultz group using combinatorial approaches [33]. Studies from different laboratories revealed that the cell cycle in cultured cancer cell lines have been

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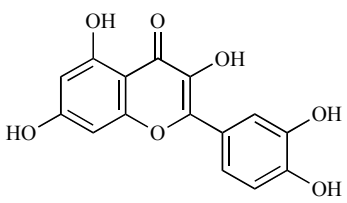
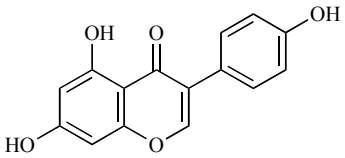
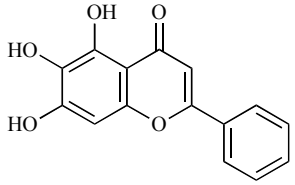


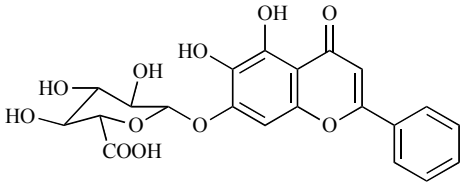
Quercetin (**1**)
CDK-4/cyclin D1 IC_{50}: 61 μM
CDK-2/cyclin B1 IC_{50}: 20 μM



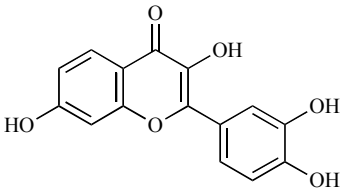
Genistein (**2**)
CDK-4/cyclin D1 IC_{50}: 250 μM
CDK-2/cyclin B1 IC_{50}: 370 μM



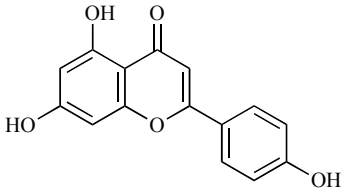
Baicalein (**3**)
CDK-1/cyclin B IC_{50}: 6.5 μM



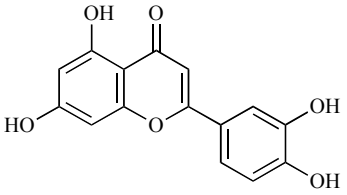
Baicalin (**4**)
CDK-1/cyclin B IC_{50}: 14.4 μM



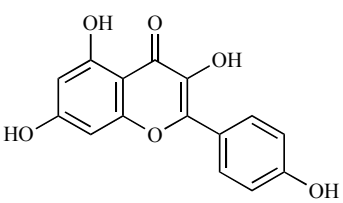
Fisetin (**5**)
CDK-1/cyclin B IC_{50}: 0.79 μM
CDK-5/p25 IC_{50}: 0.57 μM
CDK-6/cyclin V IC_{50}: 0.85 μM



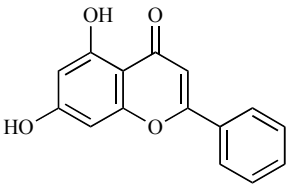
Apigenin (**6**)
CDK-1/cyclin B IC_{50}: 4 μM
CDK-5/p25 IC_{50}: 1.6 μM
CDK-6/cyclin V IC_{50}: 1.7 μM



Luteolin (**7**)
CDK-1/cyclin B IC_{50}: 6.2 μM
CDK-5/p25 IC_{50}: 3.8 μM
CDK-6/cyclin V IC_{50}: >300 μM



Kaempferol (**8**)
CDK-1/cyclin B IC_{50}: 41 μM
CDK-5/p25 IC_{50}: 51 μM
CDK-6/cyclin V IC_{50}: 22 μM



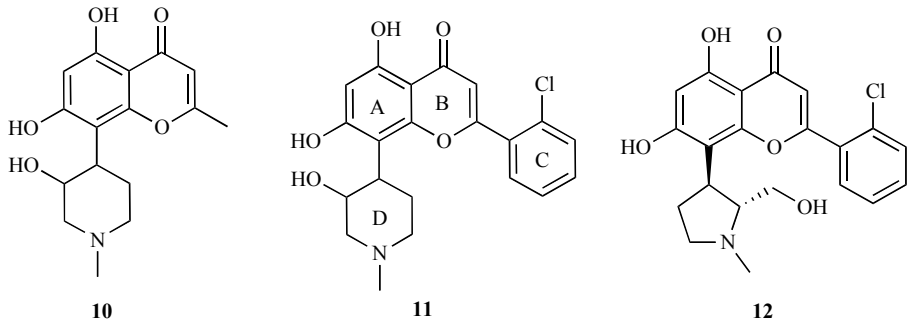
Chrysin (**9**)
CDK-1/cyclin B IC_{50}: 7 μ.1M
CDK-5/p25 IC_{50}: 3.1 μM
CDK-6/cyclin V IC_{50}: 6 μM

Fig. (1). Naturally occurring CDK inhibitory flavonoids

found to be perturbed by flavonoids such as silymarin, genistein, quercetin, daidzein, luteolin, kaempferol, apigenin, and epigallocatechin 3-gallate [11]. Flavonoids like quercetin (**1**), genistein (**2**), baicalein (**3**), baicalin (**4**), fisetin (**5**), apigenin (**6**), luteolin (**7**), kaempferol (**8**) and chrysin (**9**) have shown CDK inhibitory activity as mentioned in Fig. (**1**) [34, 35]. Baicalein and quercetin are also reported to inhibit cell proliferation in several types of cells. They mediated G1 and G2 growth arrest accompanied by the down-regulation of cyclin D2, cyclin A, CDK1 and CDK2 [36, 37]. Zhang *et al*. synthesized series of nitrogen-containing baicalein, quercetin and chalcone analogues and evaluated their CDK1 activity [35].

Like flavonoids, alkaloids is another largest group of secondary metabolites which widely occurs in terrestrial as well as marine organisms. Chemically it contain usually basic (in some cases neutral or quaternary) nitrogen derived from an amino acid in a heterocycle. One of the very important group of alkaloids is chromone and flavonoid alkaloids. This group of alkaloids are of interest not only due to their amphoteric (being both bases and phenols) nature, but because of the pronounced biological activity of some of the natural sources which contain them. A structure consisting of a nitrogen system (such as pyridine, piperidine, pyrrolidine) linked to the 'A' ring of chromone nucleus (5,7-dihydroxy-2-methylchromone; noreugenin) is referred to as a chromone alkaloids; whereas structures consisting of a nitrogen system linked to the 'A' ring of chromone and bearing an aryl substituent at *C*-2 are called as flavonoid alkaloids or 'flavoalkaloids'.

Large number of naturally occuring chromone alkaloids and flavoalkaloids have been reported in the literature, varying in type of nitrogen system and its position of attachment on chromone/flavonoid nucleus. Their natural occurrence and biological activities have been recently reviewed by Khadem and Marles [38]. Rohitukine (**10**) is a chromone alkaloid originally isolated from the stems and leaves of *Ammora rohituka* Roxb. (Family: Meliaceae) [39]. Later it was isolated by chemists at Hoechst India Ltd. in the early 1990s from *Dysoxylum binectariferum* Hook. which is phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd. used for treatment of rheumatoid arthritis. Rohitukine (**10**) was isolated as the constituent responsible for anti-inflammatory and immunomodulatory activity [40]. Medicinal chemistry efforts around this nature-derived chromone alkaloid led to discovery of two promising clinical candidates for treatment of cancer *viz*. flavopiridol (**11**) and P-276-00 (**12**), acting via inhibition of cyclin-



10 **11** **12**

Fig. (2). Structures of rohitukine (**10**), flavopiridol (**11**) and P-276-00 (**12**).

dependent kinases. Flavopiridol (**11**) has emerged in the late 1980s from a natural product (rohitukine) based drug discovery research programme at Hoechst India Limited as an EGFR inhibitor. P-276-00 (**12**) was discovered by Piramal Life Sciences India from their flavopiridol based anticancer drug discovery program. Chemical structures of **10-12** are shown in Fig. (**2**).

Recently Wang and Ren (2010) [41] reviewed combination chemotherapy aspects of flavopiridol. However, there is no any comprehensive review appeared on chemistry and pharmacology of CDK inhibitory flavoalkaloids. The present article provides detailed account on natural product chemistry, total synthesis, medicinal chemistry, in vitro and preclinical pharmacology and clinical studies of flavoalkaloids as CDK inhibitors. The article has been divided into four sections: (a) synthesis, (b) medicinal chemistry, (c) preclinical pharmacology and clinical studies and (d) computational studies and SAR.

2. SYNTHESIS

First total synthesis of rohitukine (**10**) [40] and flavopiridol (**11**) [42] was reported by Hoechst researchers in 1990s as depicted in Fig. (**3**). Tabaka *et al.* (1999) [43] provided an improved version of this synthetic strategy for racemic flavopiridol and its salt. The first step involves condensation of 1,3,5-trimethoxybenzene (**13**) with 1-methyl-3-piperidinone (**14**) in presence of acetic acid saturated with hydrogen chloride leading to formation of olefin **15**, which on hydroboration produced trans-arylpiperidinol (trans-**16**). Required cis-arylpiperidinol (cis-**16**) was obtained by inversion of the alcohol stereochemistry in two steps: Swern oxidation of trans-**16** to the corresponding ketone **17** followed by reduction with sodium borohydride. The reduction product was a mixture of cis/trans alcohols (cis-**16** and trans-**16**) (present in a 7:3 ratio as determined by GC analysis). Resolution of cis/trans alcohol was achieved either by fractional crystallization of its diastereomeric salts with (-)-dibenzoyl-D-tartaric acid, or by separation (silica gel flash chromatography) of its diastereomeric ester mixture with (-)-menthyloxyacetic acid. Acylation of cis-**16** using acetic anhydride in the presence boron trifluoride etherate gave **18** in 73% yield. Saponification of **18** provided *O*-hydroxyacetophenone intermediate **19** in which OAc gets deprotected. Compound **19** was subjected to reaction with ethyl acetate and sodium metal, whereby the chromone ring was formed. The product **20** was demethylated by heating with pyridine hydrochloride and a small amount of quinoline to get rohitukine (**10**). This total synthesis offers the flexibility to prepare a variety of structural analogues for SAR studies. Flavopiridol (**11**) and P276-00 (**12**) and other analogues were synthesized by same method with minor modifications. For flavopiridol (**11**), benzoylation of **18** using 2-chlorobenzoyl chloride provided the benzoate **21** in 76% yield. The ortho substitution between the benzoate functionality and the acetophenone set up a flavone-forming step. Treatment of **21** with KOH in pyridine at reflux gave rearranged intermediate, which on treatment with AcOH/ H_2SO_4 undergo dehydration to provide **22**. Deacetylation followed by demethylation of resulting product gave flavopiridol (**11**).

Kim *et al* [44] have done minor modification for synthesis of oxo- and thio-flavopiridol analogues **23-29**: resolution of recemic ketone (**17**) by dynamic kinetic resolution. Heating of racemic piperidone **17** in the presence of dibenzoyl-D-tartaric acid in methanol provided optically pure piperidone (**17**) in 76% yield. Reduction of optically pure piperidone **17** using DIBAL-H provided the mixture of *cis*-**16** (56% yield) and *trans*-**16** (13% yield). Cis-**16** further on acetylation followed by treatment with carbon disulfide produced thiol **30**. Further ethylation of **30** followed by oxidation using mCPBA led to formation of sulphone **32**. Treatment of sulphone **32** with phenol/ thiophenol followed by demethylation resulted in formation of corresponding oxo- **29** or thioflavopiridols **23-28**. Similarly aminoflavopiridol analog **34** was prepared from **31** by treatment with aniline (Fig. **4**).

Schoepfer *et al* [45] synthesized benzofuranone derivatives **35-39** starting from dimethoxy phenol **40** and 1-methyl-4-piperidone (**14**) as depicted in Fig. (**5**). Acid-catalyzed condensation of **40** with **14** afforded the unsaturated derivative **41** in 62% yield. The compound **41** was then hydrogenated to afford **42**, which was treated successively with chloroacetyl chloride and aluminum chloride in a one-pot procedure to afford the benzofuranone derivative **43** in 50% yield. Benzofuranone **43** further on condensation with different benzaldehyde derivatives followed by demethylation led to formation of analogs **35-39**.

Piramal recently disclosed a new process (Fig. **6**) for the preparation of pure P276-00 (**12**) pyrrolidino-flavone enantiomer based on optimized resolution of a synthetic

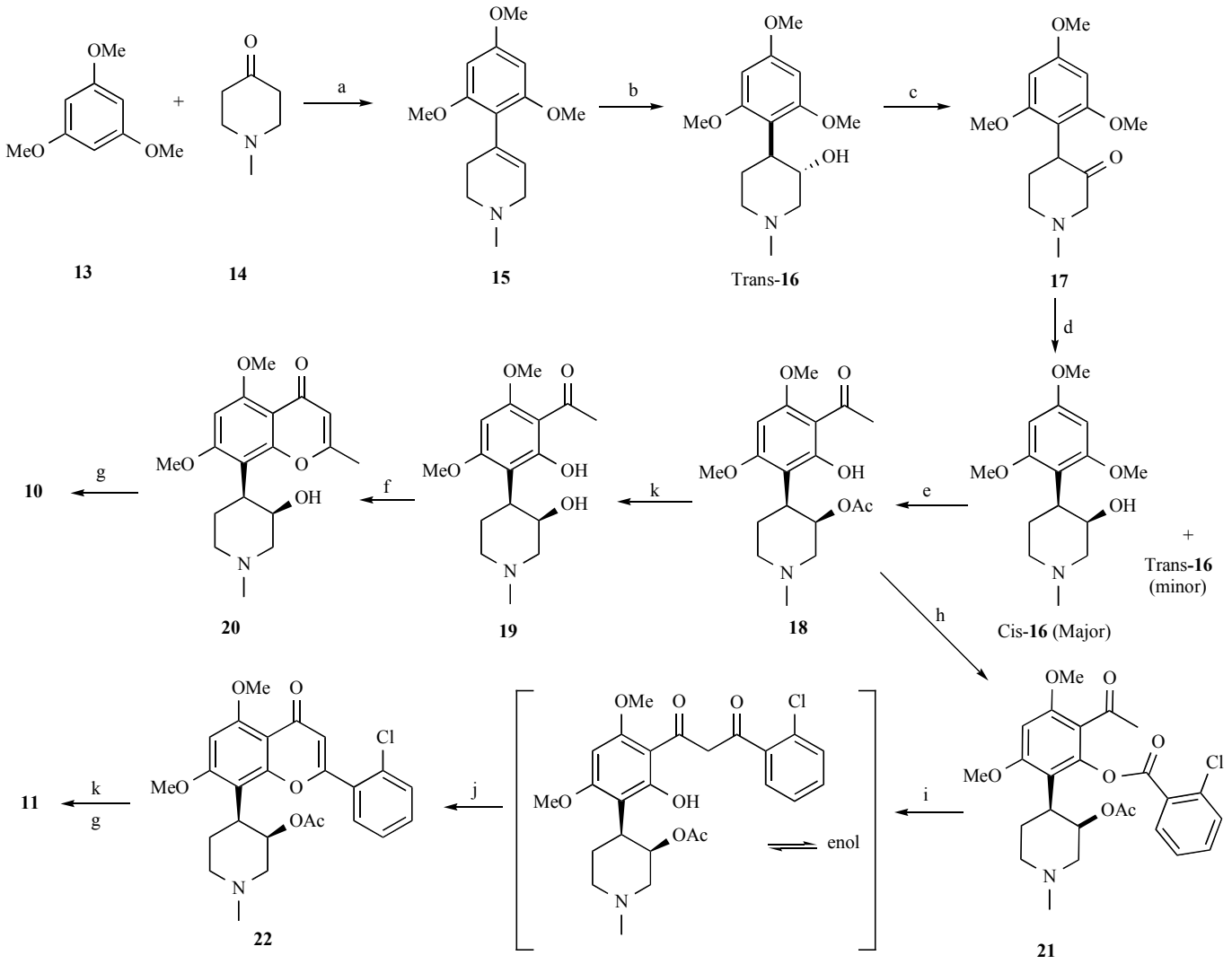


Fig. (3). Scheme for synthesis of rohitukine (**10**) [40] and flavopiridol (**11**) [42, 43]. Reagent and conditions: (a). CH_3COOH, HCl, 100 °C, 3 h, 80%; (b). BF_3-OEt, $NaBH_4$, THF, conc. HCl, then NaOH, H_2O_2, 70%; (c). $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 76%; (d). $NaBH_4$, EtOH, 66%; (e). BF_3-OEt, Ac_2O, CH_2Cl_2, 73%; (f). EtOAc, Na, reflux, 3 h; (g). pyridinium HCl, quinoline, 180 °C, 2 h, 82%; (h). 2-Cl-benzoylchloride, pyridine, 76%; (i). KOH, pyridine, reflux; (j). AcOH/ H_2SO_4; (k). NaOH/ H_2O, MeOH, 82% from **21**.

intermediate prior to formation of the pyrone ring [46]. Michael addition of dimethyl malonate (**46**) to (E)- methyl-2-nitro-3-(2,4,6-trimethoxyphenyl) acrylate (**45**) in chloroform in the presence of a indeno-oxazole based catalyst complex, a base and molecular sieves resulted in formation of (+)-trimethyl 3-nitro-2-(2,4,6-trimethoxyphenyl) propane-1,1,3-tricarboxylate (**47**) in 69% yield. Reduction of **47** with stannous chloride gave **48**, which further on decarboxylation led to formation of (+)-methyl-5-oxo-3-(2,4,6-trimethoxy-phenyl)pyrrolidine-2- carboxylate (**49**) as a mixture of cis and trans isomers. (-)-Trans-1-methyl-5-oxo-3-(2,4,6-trimethoxyphenyl)- pyrrolidine-2-carboxylic acid (**50**) was obtained by N-methylation of **49**. Hydrolysis of ester **50** followed by reduction of resulting acid with LAH/THF gave a key intermediate **52** with a chiral purity of 97-99% ee (enantiomeric excess). Further this intermediate **52** was converted to P-276-00 (**12**) using similar procedure as reported for flavopiridol synthesis in Fig. (**6**).

3. MEDICINAL CHEMISTRY

Extensive amount of medicinal chemistry efforts around rohitukine (**10**) have been reported, which led to discovery of flavopiridol (**11**) and P-276-00 (**12**). Flavopiridol is the first CDK inhibitor to enter clinical trials and this falls under the class of first generation CDK inhibitors. Several derivatives have been synthesized to determine a structure–activity relationship. We have divided different modifications and corresponding analogs into three major classes *viz*. ring C modifications (type I), ring B modifications (type II) and ring D modifications (type III) as depicted in Fig. (**7**).

C-Ring Modifications (Type I)

Ali *et al.* have prepared a series of C-ring modified analogs of type Ia **53-68** (chiral flavopiridol analogs) and analogs of type Ic **69-72** (analogs of D-ring olefin flavopiridol, which lacks the ring D hydroxyl) and evaluated for anti-HIV activity. The human positive transcription

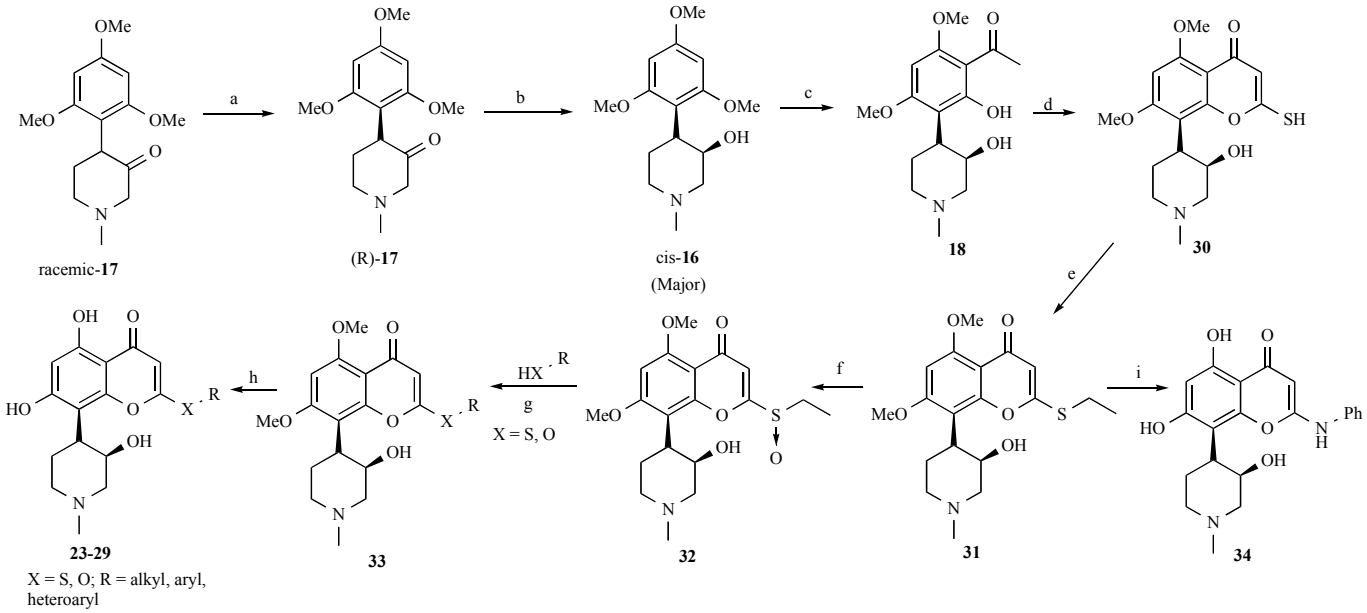


Fig. (4). Scheme for synthesis of oxo-, thio- and amino-flavopiridol analogs **23-29** and **34** by Kim *et al* [44]. Reagent and conditions: (a). Dibenzoyl-D-tartaric acid, MeOH, 25-85 °C,76%; Aq. NaOH, 99%; (b). DIBAL-H,-76 °C, DCM, 3.5 h, 99%; (c). BF_3-OEt, Ac_2O, DCM, 0 °C, 82%; (d). CS_2, LHMDS, THF, 2 h, rt, 79%; (e). $CsCO_3$, C_2H_5I, DMF, 18 h, rt, 98%; (f). mCPBA, DCM, TFA, 0 °C, 1 h, rt, 96%; (g). KOtBu, THF, 2-chloro-thiophenol, 90%; (h). BBr_3, DCM, 80 °C, 4h, 68%; (i). aniline, rt, 46%.

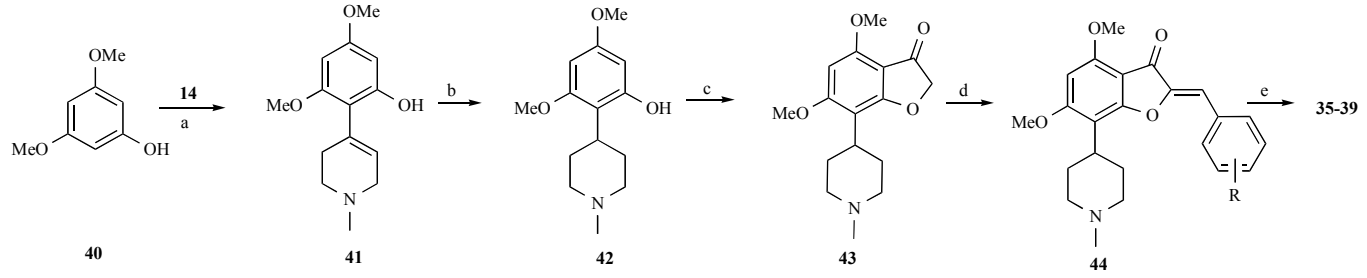


Fig. (5). Scheme for synthesis of benzofuranone derivatives **35-39** by Schoepfer *et al.* [45]. Reagent and conditions: (a). HCl gas, acetic acid, 25 °C, 24 h, 62%; (b). H_2, Pd/C, acetic acid-water, 25 °C, 20 h, 87%; (c). chloroacetyl chloride, $AlCl_3$, 0-100 °C, 1 h, 50%; (d). KOH, EtOH, 25 °C, 1 h; (e). pyridinium hydrochloride, 180 °C, 3 h, 50%.

elongation factor (P-TEFb), which is composed of CDK-9/cyclin T1 regulates RNA polymerase II-dependent transcription of cellular and integrated viral genes. It is an essential cofactor for HIV-1, selective inhibition of P-TEFb blocks HIV-1 replication without affecting cellular transcription; this indicates that P-TEFb could be a potential target for developing anti-HIV-1 therapeutics. All compounds were potent inhibitors of CDK-9/cyclin T1 with IC_{50} values in nanomolar concentrations [47]. Kim *et al.* from BMS research Institute have synthesized series of analogs bearing a heteroatom linker between ring B and C. This includes sulphur linked analogs **23-28**, oxo- **29** and aminoflavopiridols **34** (Type Ib analogs) as well C-ring modified flavopiridol analog **73** (Type Ia analog). Introduction of a sulfur and an oxygen linker increases the selectivity towards CDK-1/B over CDK-2/E and CDK-4/D1 (Table 1). However, the enhanced selectivity is only a result of the reduced affinity [44]. Murthi *et al.* synthesized type Ic analogs **72, 74-82** and evaluated their effect on CDK-1 and CDK-4 inhibitory activity to determine a structure-activity relationship. A 2-bromo substituted analog **77** showed potent inhibition of CDK1 and CDK4 with IC_{50} values of 0.98 and 0.65 μM respectively [48]. The 4-fluorophenyl analog **57** is more potent inhibitor of CDK2 than flavopiridol (**11**). The SAR of flavopiridol C-ring analogs (type Ia) resembles closely to the SAR of olefin analogs (Type Ic) [48]. Activities of type I analogs are summarized in Table **1**.

B-Ring Modifications (Type II)

Replacement of B-ring oxygen with nitrogen (analog **83**, type IIa) or changing the position of oxygen in the B ring (analog **84**, type IIb) resulted in decrease in CDK-4 inhibitory activity. CDK-4 IC_{50} value of parent molecule **79** was 1 μM, however pyridine-4-one analog **83** and pyran-2-one analog **84** showed IC_{50} values of 44 and 23 μM respectively [48]. B-ring constrained analogs in which the 5-hydroxychromone skeleton of flavopiridol (**11**) has been replaced by 4-hydroxybenzofuranone skeleton (type IIc analogs **35-39**) have been reported. The most potent compound was the sulfonamide **38** showing CDK1 and CDK2 inhibition at IC_{50} of 9 and 30 nM respectively. The introduction of a chloro substituent in the ortho position of

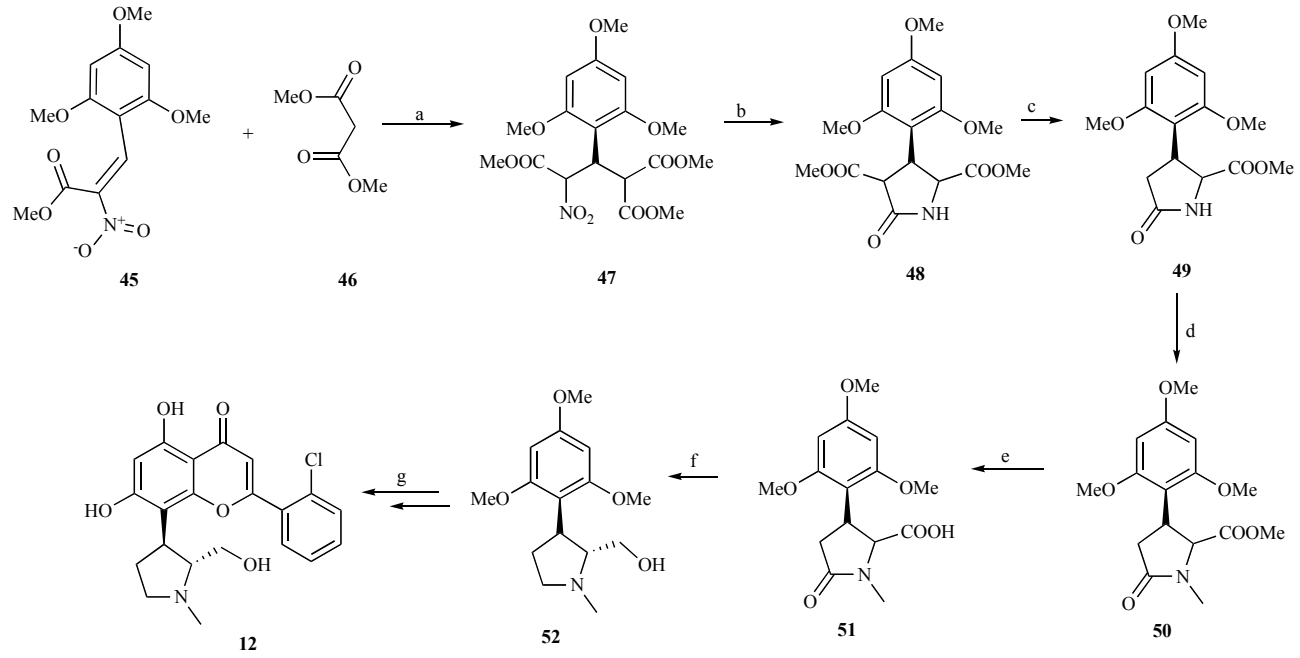


Fig. (6). Scheme for synthesis of P-276-00 (**12**) by chemists from Piramal Life Sciences [46]. Reagent and conditions: (a). N-methylmorpholine, catalyst complex, $CHCl_3$, H_2O, Mol. sieve,12 h, rt, 69%; (b). $SnCl_2$, ethyl acetate, 55 °C, 2 h, 67%; (c). N-methylpyrrolidone, NaCl, H_2O, 170 °C, 5 h, 44%; (d). NaH, CH_3I, DMF, 0 °C to rt, 1 h, 95%; (e). KOH, MeOH, H_2O, 65 °C, 3 h, 61%; (f). $LiAlH_4$, THF, 50 °C. 1.5 h, 100% ; (g). steps e, h, i, j. g from Fig. (**3**).

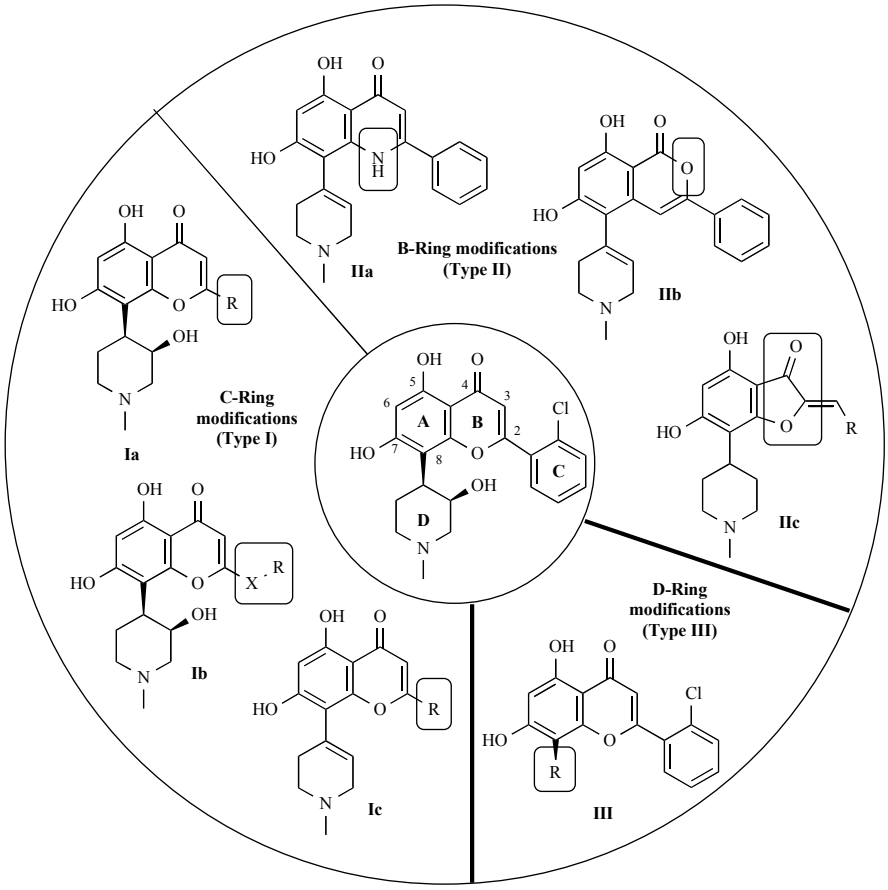


Fig. (7). Medicinal chemistry around rohitukine (**10**)/flavopiridol (**11**). Three main types of analogs have been reported. These include C-ring modifications (Type **I**); B-ring modifications (Type **II**) and D-ring modifications (Type **III**).

Table 1. CDK Inhibitory Activity of Flavopiridol (11) and Type I[a] (C-ring Modifications) Analogs

Entry (Scaffold)	IC_{50} against CDK1/ CDK2/ CDK4/ CDK9 (μM)	X	R	Ref	Entry (Scaffold)	X	R	IC_{50} against CDK1/ CDK2/ CDK4/ CDK9 (μM)	Ref
11	0.03/0.17/0.10/nd	-	Ph(2-Cl)	[44]	(+)**25** (Ib)	S	2-chlorophenyl	6.1/4.4/>25/nd	[44]
53 (Ia)	nd/0.19/nd/0.009	-	Ph	[47]	(±)**26** (Ib)	S	phenyl	0.44/6.59/4.1/nd	[44]
54 (Ia)	nd/0.161/nd/0.004	-	Ph(3-Cl)	[47]	(±)**27** (Ib)	S	t-Bu	0.08/1.07/2.0/nd	[44]
55 (Ia)	nd/0.286/nd/0.012	-	Ph(4-Cl)	[47]	(±)**28** (Ib)	S	pyrimidin-2-yl	6.4/40.4/82.5/nd	[44]
56 (Ia)	nd/0.356/nd/0.0028	-	Ph(2-F)	[47]	(-)**29** (Ib)	O	2-chlorophenyl	0.13/2.11/6.15/nd	[44]
57 (Ia)	nd/0.129/nd/0.0021	-	Ph(4-F)	[47]	(±)**34** (Ib)	NH	Ph	16.3/>25/>25/nd	[44]
58 (Ia)	nd/0.223/nd/0.0055	-	Ph(4-Br)	[47]	**69** (Ic)	-	Ph (4-F)	nd/0.208/nd/0.0065	[47]
59 (Ia)	nd/0.567/nd/0.019	-	Ph(4-tBu)	[47]	**70** (Ic)	-	Ph (2-Br)	nd/0.638/nd/0.0054	[47]
60 (Ia)	nd/0.301/nd/0.018	-	Ph(4-CF3)	[47]	**71** (Ic)	-	pyridin-3-yl	nd/1.02/nd/0.012	[47]
61 (Ia)	nd/0.196/nd/0.009	-	Ph(4-OH)	[47]	**72** (Ic)	-	Ph (2-Cl)	1.1/468.3/0.88.7	[47, 48]
62 (Ia)	nd/0.886/nd/0.011	-	Pyridin-2-yl	[47]	**74** (Ic)	-	Ph (4-Cl)	1.7/nd/5.5/nd	[48]
63 (Ia)	nd/0.247/nd/0.005	-	Pyridin-3-yl	[47]	**75** (Ic)	-	Ph (3-Cl)	1.2/nd/2.8/nd	[48]
64 (Ia)	nd/0.208/nd/0.006	-	Pyridin-4-yl	[47]	**76** (Ic)	-	Ph (2-F)	2.3/nd/1.8/nd	[48]
65 (Ia)	nd/0.314/nd/0.013	-	Pyridin-3-yl (2-Cl)	[47]	**77** (Ic)	-	Ph (2-Br)	0.98/nd/0.65/nd	[48]
66 (Ia)	nd/0.238/nd/0.019	-	Isoxazol-3-yl (5-Me)	[47]	**78** (Ic)	-	Ph (2-I)	na/nd/2.5/nd	[48]
67 (Ia)	nd/0.129/nd/0.009	-	Ph (3-allyl)	[47]	**79** (Ic)	-	Ph	nd/nd/1.0/nd	[48]
68 (Ia)	nd/0.206/nd/0.009	-	Ph (4-allyl)	[47]	**80** (Ic)	-	Ph (2,4-di-Cl)	nd/nd/1.2/nd	[48]
73 (Ia)	2.5/9.69/3.7/nd	-	piperidin-N-yl	[44]	**81** (Ic)	-	pyridin-4-yl	nd/nd/0.8/nd	[48]
(±)**23** (Ib)	0.46/3.93/2.06/nd	S	Et	[44]	**82** (Ic)	-	cyclohexyl	nd/nd/7.0/nd	[48]
(-)**24** (Ib)	0.11/2.1/16.2/nd	S	2-chlorophenyl	[44]					

[a]See Fig. (**7**) for type of scaffold.

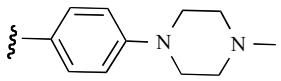
the phenyl ring does not result in an increased activity [45]. Activities of type II analogs are summarized in Table **2**.

D-Ring Modifications (Type III)

A series of type III analogs **85-90** have been reported possessing CDK-1 and CDK-4 inhibitory activity. Analog **85** showed IC_{50} values of 8 and 10 μM against CDK1 and CDK4 respectively [48]. The cis- and trans- hydroxyl isomers **86** and **87** are very poor inhibitors of CDK-4/cyclin D exhibiting IC_{50} values of 120 and 250 μM respectively, suggesting that the position of N of the D ring is very important for CDK inhibitory activity. Several D-ring replacement analogs have been reported. Replacement of ring D of flavopiridol with aryl ring such as pyridyl (compound **89**) and pyrimidyl (compound **90**) results in major loss of CDK inhibitory activity [48]. Replacement of D-ring with amide linked aryl ring analogs **91-95** also led to loss of activity. Analogs with N-linked pyrrolidine **96**, piperidine **97**, morpholine **98,** aliphatic amides **99-102**, sulphonamides **102-105**, all resulted in major loss in activity [49]. Several promising piperidine ring constrained analog of flavopiridol have been synthesized by Piramal Life Science Ltd. (India) [46]. One of the compounds, P-276-00 (**12**), was identified as potent CDK inhibitor, which selectively inhibits CDK-4/cyclin D1, CDK-1/cyclin B, and CDK-9/cyclin T1 and shows relevant antitumor activity in a broad panel of cancer-cell lines [50]. P-276-00 (**12**) is a trans-(+) enantiomer. Its trans-(-)-enantiomer **107** is less potent than parent isomer **12**. The nitro derivative of P-276-00 with the same stereochemistry, **108** was found to be slightly more potent showing CDK-2/cyclin E inhibition with IC_{50} of 1.7 μM. Activities of type III analogs are summarized in Table **3**.

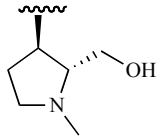
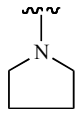
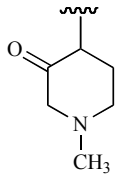
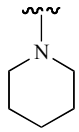
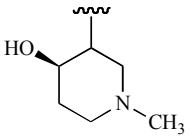
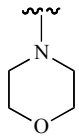
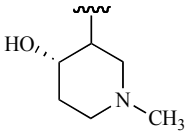
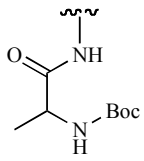
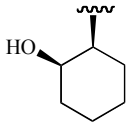
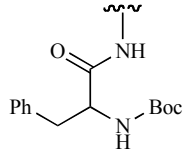
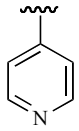
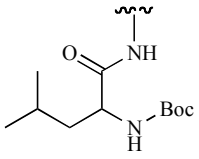
Thus, a synthetic campaign performed for structure-activity relationship studies resulted in flavopiridol (**11**), the

Table 2. CDK Inhibitory Activity of Type II[a] (B-ring Modifications)Analogs

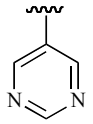
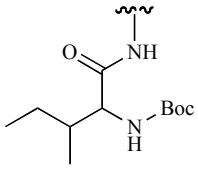
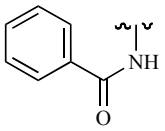
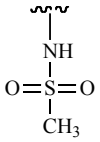
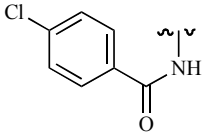
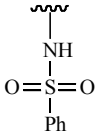
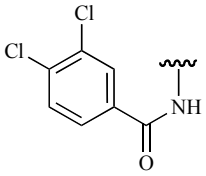
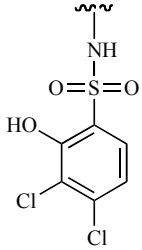
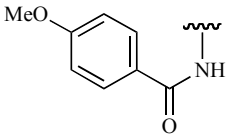
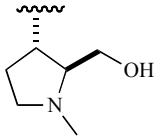
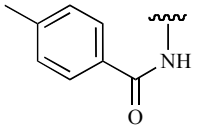
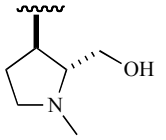
Entry (Scaffold)	R	IC_{50} (μM)				Ref
		CDK1	CDK2	CDK4	CDK9	
35 (IIc)	Ph	0.11	1.28	4.41	nd	[45]
36 (IIc)	Ph (2-Cl)	0.6	3.97	25.5	nd	[45]
37 (IIc)	Ph (4-NO_2)	0.06	0.31	2.21	nd	[45]
38 (IIc)	Ph (4-SO_2NH_2)	0.009	0.03	1.87	nd	[45]
39 (IIc)		0.08	2.98	1.92	nd	[45]
83 (IIa)[b]	-	nd	nd	44	nd	[48]
84 (IIb)[b]	-	nd	nd	23	nd	[48]

[a]see Fig. (**7**) for type of scaffold; [b]scaffold IIa = **83** and IIb = **84** (Fig. **7**).

Table 3. CDK Inhibitory Activity of Type III[ab] (D-ring Modifications) Analogs

Entry	R	IC_{50} against CDK's in μM				Ref	Entry	R	IC_{50} against CDK's in μM				Ref
		1	2	4	9				1	2	4	9	
12		nd	2.8	nd	0.02	[51]	**96**		nd	417	nd	nd	[49]
85		10	nd	8	nd	[48]	**97**		nd	417	nd	nd	[49]
86		na	nd	120	nd	[48]	**98**		nd	417	nd	nd	[49]
87		na	nd	250	nd	[48]	**99**		nd	417	nd	nd	[49]
88		12	nd	250	nd	[48]	**100**		nd	417	nd	nd	[49]
89		22	nd	21	nd	[48]	**101**		nd	417	nd	nd	[49]

(Table 3). Contd…..

Entry	R	IC_{50} against CDK's in μM				Ref	Entry	R	IC_{50} against CDK's in μM				Ref
		1	2	4	9				1	2	4	9	
90		85	nd	160	nd	[48]	**102**		nd	417	nd	nd	[49]
91		nd	417	nd	nd	[49]	**103**		nd	219	nd	nd	[49]
92		nd	339	nd	nd	[49]	**104**		nd	178	nd	nd	[49]
93		nd	217	nd	nd	[49]	**105**		nd	94	nd	nd	[49]
94		nd	54	nd	nd	[49]	**106**		nd	21	nd	nd	[51]
95		nd	90	nd	nd	[49]	**107**	 & 4-NO_2 in ring C[b]	nd	1.7	nd	nd	[51]

[a]see Fig. (**7**) for type of scaffold; [b] for structure of **107**, see Fig. (**8**); na, not active; nd, not determined.

only compound from over 100 analogues synthesized [42] and was the first CDK inhibitor to enter into human clinical trials [52]. Flavopiridol (**11**, NSC 649890, L86-8275), [cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-1-benzopyran-4-one] though it is totally synthetic molecule, the basis for its novel structure is a natural product, rohitukine (**10**). Flavopiridol (**11**) was originally identified as a micromolar inhibitor of different protein kinases such as the receptor tyrosine kinases (EGFR, IC_{50} = 21-25 μM), receptor-associated kinases (src kinases, IC_{50} = 50 μM), and cytosolic signal transduction kinases (protein kinase A, IC_{50} = 122–145 μM; PKC, IC_{50} = 6 μM; Erk-1, IC_{50} =16 μM) [53-55]. The highest activity of this compound is observed against the CDKs and the evolutionary related glycogen synthase 3β (GSK-3β, IC_{50} = 450 nM) [56]. Flavopiridol has been shown to be a potent inhibitor of cyclin-dependent kinase CDK-1, CDK-2, CDK-4, and CDK-7 [57]. The IC_{50} values of flavopiridol for CDK1, CDK2 and CDK4 are 30-40, 100, 20-40 nM respectively [54, 57, 58].

4. PRECLINICAL PHARMACOLOGY AND CLINICAL STUDIES

4.1. Flavopiridol

Preclinical Pharmacology

Flavopiridol inhibits CDKs 1, 2, and 4 in a competitive manner with respect to ATP, with a ki of 41 nM [53, 54, 57, 59]. Through inhibition of CDKs, flavopiridol induces arrest of cell growth at the G1 and G2 phases of the cell cycle [57]. Combination of flavopiridol with taxanes, histone

deacetylase inhibitors, radiation, proteosome inhibitors, monoclonal antibodies and various chemotherapeutic agents have been reported. Detailed account on these studies have been recently reviewed by Wang and Ren (2010) [41]. Flavopiridol has also been shown to enhance the activity of other growth-suppressing agents, such as tumor necrosis factor (TNF), doxorubicin, and etoposide [60-63]. Research in the last few years has indicated that besides inhibiting CDK activity, the expression of anti-apoptotic proteins, such as Bcl-2 [64], Mcl-1 [65, 66], cyclin D1 [62, 67], and vascular endothelial growth factor [68, 69] has been shown to be suppressed by flavopiridol. All of the genes for these proteins are known to be regulated by the nuclear transcription factor NF-kB. Flavopiridol has also been shown to suppress inflammation and regulate the immune system [64]. Flavopiridol inhibits NF-kB activation induced by various carcinogens and inflammatory agents through inhibition of IkBα kinase and p65 phosphorylation [70].

Another interesting aspect of flavopiridol's cell cycle regulatory properties is the depletion of cyclin D1, an oncogene that is upregulated in many human neoplasias and, when overexpressed harbors bad prognosis [71]. When MCF-7 human breast carcinoma cell lines were exposed to flavopiridol, cyclin D1 protein levels decreased within 3 hours. This effect was followed by decline in cyclin D3 with no alteration in cyclin D2 or cyclin E protein levels, the remaining G1 cyclins. Two hours later, CDK4 activity obtained from intact cells incubated with flavopiridol declined, suggesting that depletion of cyclin D1 leads to loss of CDK activity [67]. In order to determine if flavopiridol (**11**) possesses anti-angiogenic properties, Brusselbach *et al.* exposed HUVEC human endothelial cells to flavopiridol. Clear evidence of apoptosis was determined even in cells that were non-cycling [72]. In another report, Kerr *et al.* tested flavopiridol in an *in vivo* angiogenesis model; again, flavopiridol (**11**) was able to decrease blood formation in the

Table 4. Summary of Currently Ongoing Clinical Studies of Flavopiridol

Sr. No.	Combined drug(s)	Cancer type	Phase	Sponsors	Status	Clinical trial ID
1.	-	Lymphoma; Multiple Myeloma and Plasma Cell Neoplasm	I/II	Arthur G. James Cancer Hospital & Richard J. Solove Research Institute/ NCI	Unknown	NCT00112723*
2.	-	Leukemia; Lymphoma	I (genetic cytogenetic analysis)	Arthur G. James Cancer Hospital & Richard J. Solove Research Institute/ NCI	Unknown	NCT00377104*
3.	Bortezomib	Lymphoma, multiple myeloma, plasma cell neoplasm	I	NCI /Virginia Commonwealth University	Recruiting participants	NCT00082784
4.	Oxaliplatin	Extragonadal Germ Cell Tumor; Ovarian Cancer; Testicular Germ Cell Tumor	II	NCI/ Memorial Sloan-Kettering Cancer Center	Unknown	NCT00957905
5.	Lenalidomide	Leukemia; Lymphoma	I	NCI/ Arthur G. James Cancer Hospital & Richard J. Solove Research Institute	Unknown	NCT00735930
6.	Irinotecan	Adenocarcinoma of the Gastroesophageal Junction; Gastric Cancer	II	NCI/Memorial Sloan-Kettering Cancer Center	Recruiting	NCT00991952
7.	Ara-C and Mitoxantrone	Acute Myelogenous Leukemia	II	Sidney Kimmel Comprehensive Cancer Center	Recruiting	NCT01413880
8.	Cytarabine, and Mitoxantrone hydrochloride or Cytarabine and Daunorubicin	Leukemia	II	Sidney Kimmel Comprehensive Cancer Center/ NCI	Recruiting	NCT01349972
9.	Cyclophosphamide and Rituximab	Leukemia, lymphoma	I	Arthur G. James Cancer Hospital & Richard J. Solove Research Institute/NCI	Recruiting	NCT01076556
10.	Depsipeptide	Small cell carcinoma	I	NCI	Recruiting	NCT00094978

*single agent clinical trials.

mouse Matrigel model of angiogenesis [73]. Melillo *et al.* demonstrated that hypoxia-induced VEGF from human monocytes is obliterated by the presence of low nM concentrations of flavopiridol (**11**). Moreover, this effect was due to decrease in VEGF mRNA stability, with concomitant decline in VEGF protein [69].

Further studies demonstrated that flavopiridol (**11**) was not cytotoxic to stationary-phase cells but reversibly inhibited the growth of cells in exponential growth phase at concentrations of 25-160 nM. This study suggested that flavone **11** has delayed the progression of breast carcinoma cells through S phase; prevented progression through G2 and prevented the G2-related increase in histone H1 kinase activity, which corresponds with its ability to block CDK-1 and CDK-2 [53, 59]. Another study at NCI showed that flavopiridol (**11**) possessed potent growth inhibitory effect across panel of 60 cell lines, the mean IC_{50} across the panel for 48 h drug exposure was 66 nM [74]. Other study using four day exposure assay demonstrated a mean IC_{50} of 100 nM across 30 human cell lines [75]. It was noticed that, at least 16 h exposure required for maximum inhibition (one cell cycle time). In another study, using panel of 23 cell lines a mean IC_{50} of 18 nM was observed with three prostate cell lines and one melanoma cell line relatively more sensitive to remainder [76].

In leukemic cell lines and in human chronic lymphocytic leukemia (CLL) cells, *in vitro* flavopiridol (**11**) effectively induces apoptosis at clinically achievable concentrations and also decreases expression of Mcl-1 and XIAP, proteins that mediate resistance to apoptosis in CLL cells [77]. In xenograft models, the most pronounced antitumor effects were seen after prolonged exposure to Flavopiridol, prompting the evaluation of a 72-h i.v. continuous infusion every 2 weeks schedule in two phase I trials in humans [78]. Moreover, preclinical studies demonstrated the capacity of flavopiridol to induce programmed cell death, promote differentiation, inhibit angiogenic processes and modulate transcriptional events. Preclinical data indicated that flavopiridol (**6**) could block the proliferation of neoplastic cells and induce apoptosis as a single agent [79, 80]. Biologically active plasma concentrations of flavopiridol (300–500 nM) are easily achievable in patients receiving infusional flavopiridol.

Clinical Studies as a Single Agent

The clinical data of flavopiridol (**11**) has been reviewed by several researchers [41, 52, 58, 81, 82]. Total of 50 clinical studies (17 single and 31 combination) have been completed/terminated so far with currently 10 studies (2 single, 8 combination) are ongoing. Most of these studies are in combination regimes and only few studies (19 out of total 60) as single agent. The list of ongoing clinical trials for flavopiridol as a single/ combination regimes is provided in Table **4**.

Despite promising preclinical results, clinical activity observed with flavopiridol has been generally disappointing in a variety of solid tumors when used as a single agent [83, 84]. Single-agent flavopiridol induces responses in heavily pretreated CLL patients with unfavorable cytogenetics. The explanation for that may be related to its pharmacokinetic properties and the difficulty of defining an appropriate administration schedule with an acceptable toxicity profile. Severe side effects such as diarrhea, asthenia and serious vascular thrombotic events have also been noticed in phase II studies. Toxicity was the main concern as four patients died of infection [85].

Flavopiridol (**11**) has been studied in a wide range of indications using a variety of dosing schedules, but promising results have only emerged in treatment of chronic lymphocytic leukemia (CLL) [86, 87]. These studies indicated that unusually high plasma concentrations of flavopiridol may be required for efficacy due to high plasma protein binding [88]. It is likely that efficacy of flavopiridol (**11**) in CLL reflects effects on transcription and other targets rather than inhibition of CDKs [89, 90]. Recent clinical results, however, indicate that flavopiridol may still find niche utilities as single-agent therapy employing finely tailored dosing schedules for particularly susceptible cancers, such as chronic lymphocytic leukemia (CLL). However, in Europe, flavopiridol has already got the status of orphan drug for treatment of CLL. Currently two phase I studies (clinical trial IDs: NCT00112723 and NCT00377104) are ongoing for flavopiridol as a single agent for the treatment of lymphoma. First study is in patients with relapsed or refractory lymphoma or multiple myeloma while later one in patients with B-cell chronic lymphocytic leukemia or small lymphocytic lymphoma.

Clinical Studies in Combination Regimes

The concept of combining chemotherapeutic agents to increase cytotoxic efficacy has evolved greatly over the past several years. The rationale for combination chemotherapy has centered, in the past, on attacking different biochemical targets, overcoming drug resistance in heterogeneous tumors, and by taking advantage of tumor growth kinetics with increasing the dose-density of combination chemotherapy. The overall goal in developing combination therapies of flavopiridol was to improve its clinical efficacy with acceptable clinical toxicity. Although phase I studies of flavopiridol showed safety and some encouraging clinical activity, single agent phase II trials in solid tumors have been disappointing [87, 91, 92]. In contrast, robust significant single agent activity with tumor lysis syndrome has been observed in chronic lymphocytic leukemia, especially when the schedule of administration is altered to improve the drug's pharmacokinetic [93].

Recent emerging data revealed that flavopiridol (**11**) can potentiate, generally in a dose- and sequence-dependent manner, the anti-tumor effects of many established chemotherapeutic agents [94, 95]; which may helpful in combination with various therapeutic agents that are in or near clinical development [41]. Bible and Kaufmann [96] studied the effect of combining flavopiridol with different antineoplastic agents such as cisplatin, paclitaxel, cytarabine, topotecan, doxorubicin, etoposide, cytarabine or 5-fluorouradil in four different administration schedules in A549 human non-small cell lung carcinoma cells *in vitro.* Cisplatin was the only agent that resulted in sequence-independent synergy when combined with flavopiridol. For paclitaxel, cytarabine, topotecan, doxorubicin, and etoposide,

synergy was more pronounced when the agents were administered before flavopiridol rather than concomitant with or following flavopiridol. Similarly administration of flavopiridol for 24 h followed 3 days later by exposure to an S phase-active agent (cytarabine or 5-fluorouradil) resulted in a highly synergistic interaction. Herceptin (trastuzumab,) is a monoclonal antibody and is used as a gene-specific treatment in breast cancer. When Herceptin and flavopiridol were tested together, showed synergistically cytotoxic activity against erbB2-positive breast cancer cell lines. Addition of flavopiridol to Herceptin synergistically lowered erbB2 levels in these cells [63]. Flavopiridol and trastuzumab yield cytotoxic synergy in human breast cancer cells overexpressing Neu [62]. Cartee *et al* (2001) [97] examined interactions between flavopiridol and phorbol 12-myristate 13-acetate (PMA) in U937 human leukemia cells in relation to differentiation and apoptosis. Flavopiridol (**11**) modulates the expression/activity of multiple signaling and cell cycle regulatory proteins in PMA-treated leukemia cells and that such alterations are associated with mitochondrial damage and apoptosis rather than maturation. These observations also raise the possibility that combining CDK inhibitors and differentiation-inducing agents may represent a novel antileukemic strategy. Co-administration of bryostatin 1 (or phorbol 12-myristate 13-acetate) with flavopiridol induced a marked increase in apoptosis in U937 cells ectopically expressing an NH2-terminal phosphorylation loop-deleted Bcl-2 protein, which are otherwise highly resistant to flavopiridol-mediated lethality. Synergistic induction of apoptosis by bryostatin 1 and flavopiridol (**11**) does not stem from disruption of the leukemic cell maturation process but instead results from enhanced release of TNF-α and activation of the extrinsic apoptotic cascade, culminating in cell death [61]. Combination treatment of flavopiridol with tumor necrosis factor-α or TNF-related apoptosis-inducing ligand induced a rapid and eminent apoptosis in a human non-small cell lung carcinoma cell line indicating a synergistic effect [60].

Flavopiridol (**11**) and docetaxel have shown synergy both preclinically and in the clinical setting, the schedule of this doublet therapy appears to be critical with the optimal sequence being docetaxel followed by Flavopiridol [98-100]. This synergistic effect has also been demonstrated with the sequential administration of irinotecan followed by Flavopiridol [101]. Two pancreatic cancer patients previously treated with gemcitabine achieved significant clinical responses (1 complete and 1 partial) [102]. However, a recent phase II study testing sequential therapy with docetaxel and Flavopiridol in gemcitabine-refractory pancreatic cancer did not show any activity [103]. There is also clinical data showing that the combination of Flavopiridol with other cytotoxic agents, such as irinotecan or platinum compounds, is feasible but efficacy is limited [101, 104]. It might be worth exploring additional combination regimens with other targeted therapies whose mechanisms of action regards angiogenesis. The combination of flavopiridol with FOLFIRI (which contains folinic acid, fluorouracil and irinotecan; a chemotherapy regimen used in colorectal cancer) also showed encouraging results in phase I clinical studies of colorectal cancer. flavopiridol increases irinotecan- and fluorouracil-induced apoptosis. Clinical activity is encouraging and includes prolonged stable disease in patients with irinotecan-refractory colorectal cancer[105].

Results of one of the currently completed phase I combination study with Bortezomib conducted to determine the dose-limiting toxicities (DLT) and maximum tolerated dose in patients with B-cell malignancies (multiple myeloma, indolent lymphoma, and mantle cell lymphoma) has been published. Results indicated that the combination of bortezomib and alvocidib is tolerable and an MTD has been established for the tested schedule. The regimen appears active in patients with relapsed and/or refractory multiple myeloma or non–Hodgkin's lymphoma, justifying phase II studies to determine the activity of this regimen more definitively [106].

4.2. P-276-00 (12)

A Piramal compound P-276-00 (**12**) selectively inhibits CDK-4/cyclin D1, CDK-1/cyclin B, and CDK-9/cyclin T1 and shows relevant antitumor activity in a broad panel of cancer-cell lines [50]. P276-00 competes with ATP for binding at the catalytic site of the CDK-4 enzyme and thus inhibiting its kinase activity. The flavone P276-00 (**12**) was identified as 40-fold selective toward CDK-4/cylin D1, compared with CDK-2/cyclin E [50]. The compound **106** is a trans-(-)-enantiomer of P-276-00 (**12**, trans +) is less potent than parent isomer. P-276-00 (**12**) was ~10 times more potent against CDK-2/cyclin E than **106** (**12**, IC_{50} = 2.8 μM; **106**, IC_{50} = 21 μM). The nitro derivative **107** with the same stereochemistry as P-276-00 (**12**) was slightly more potent against CDKs. The IC_{50} of compound **107** against CDK-2/cyclin E is 1.7 μM. However, compound **107** is less potent in cellular assays (HT-29 cell line: **12**, IC_{50} = 0.58 μM; **107**,

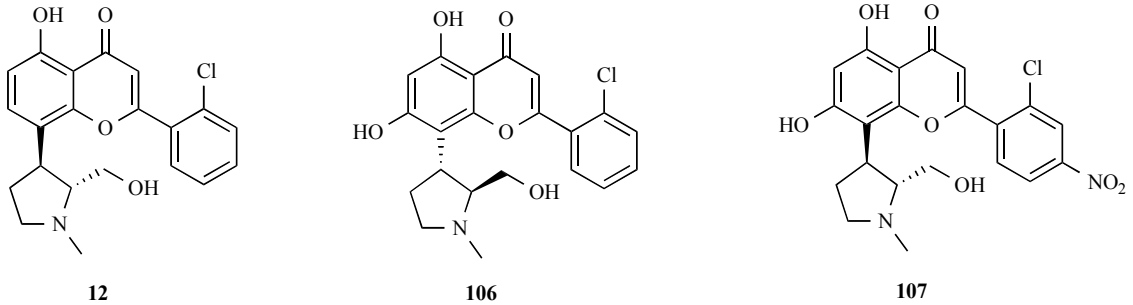


Fig. (8). Structure of P-276-00 (**12**) and its analogs **106-107**.

IC_{50} = 1.2 μM). Analog **106** also showed anti-proliferative activity in HT-29 tumor cell line with an IC_{50} of 17.9 μM. The preclinical evaluation of P-276-00 [51] and the results of Phase I clinical trials [107] revealed that interesting selectivity of the parent compound flavopiridol is maintained, particularly its potency against CDK-9/cyclin T1 (P-276-00: IC_{50} = 20 nM). Chemical structures of analogs **106** and **107** are shown in Fig. (**8**).

P276-00 (**12**) is active in human colon and non-small cell lung cancer xenografts [108]. Results of first-in human study of P276-00 has been reported [107]. The drug is administered intravenously as a 30 min i.v. infusion on day 1 to 5, and day 8 to 12, every 3 weeks. At doses below 17.6 mg/m^2 treatment was generally well tolerated, with the main adverse events being fatigue, nausea, hypotension, sweating and dry mouth, mostly grade 1 and 2. Piramal has also disclosed a synergistic effect of P-276-00 (**12**) with gemcitabine, docetaxel, paclitaxel and doxorubicin [109, 110]. This effect was observed in a cytotoxicity assay for cell lung carcinoma H-460. A parallel study involving the use of a combination comprising flavopiridol (**11**) and doxorubicin resulted in an additive effect, with no observed synergy. The synergistic effect of P-276-00 with the same antitumor agents was further established in a clonogenic assay and a xenografted mice model, using the same H-460 cell line. Currently P-276-00 (**12**) is in phase II clinical studies for advanced refractory neoplasms and multiple myeloma.

5. COMPUTATIONAL STUDIES AND SAR

The selectivity of flavopiridol (**11**) for distinct CDKs over other kinases is revealed by the crystal structure of CDK-2 in complex with the des-chloroflavopiridol (**53**). This co-crystal structure reveals several key interactions [111]. The CDK-2 interactions with ATP are characterized by predominantly hydrophobic and van-der waals interactions,

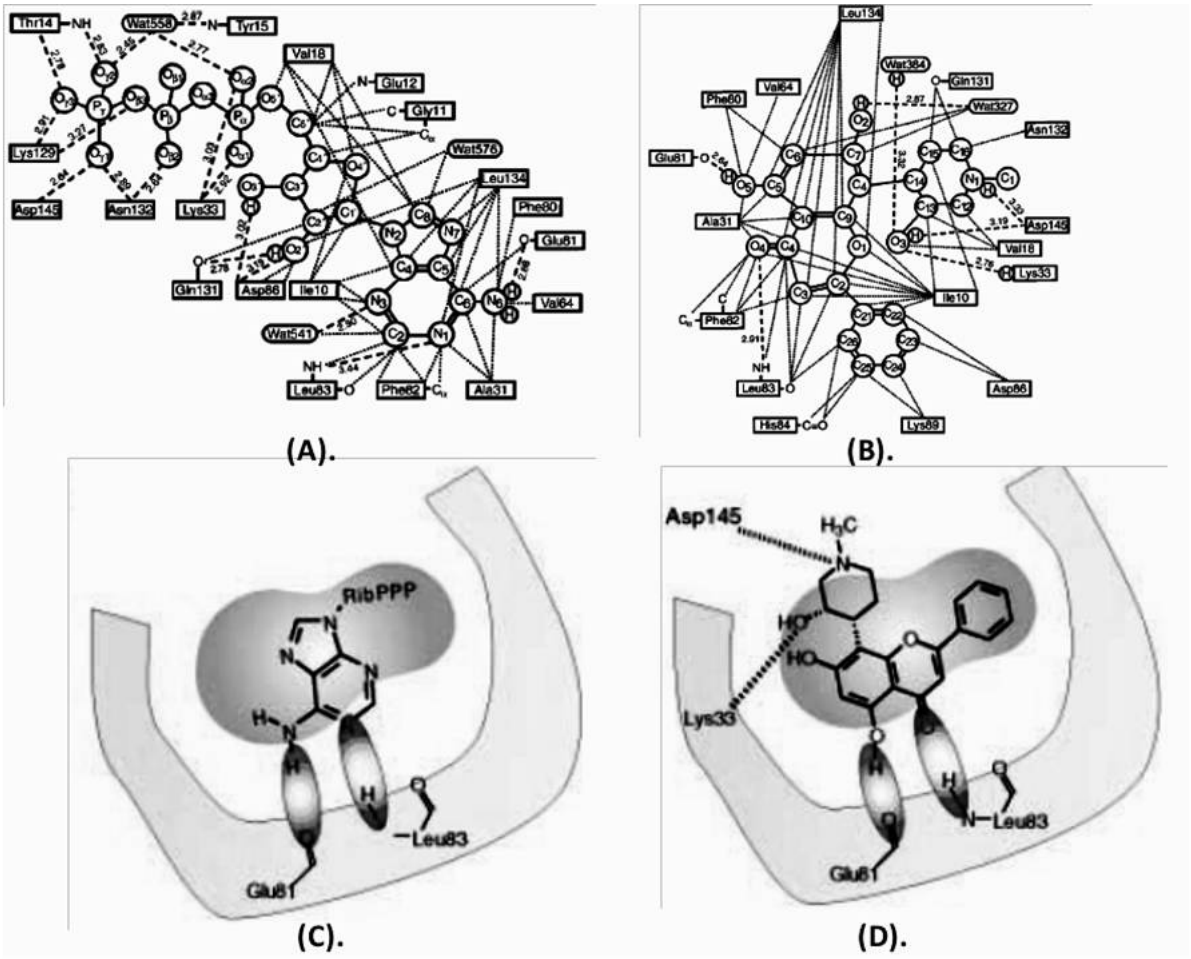


Fig. (9). (**A**). All atomic interactions between CDK2 and ATP. Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. Van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines. ATP van der Waals contacts to phosphates were omitted for clarity; (**B**). All atomic interactions between CDK2 and des-chloroflavopiridol (**53**). Contacts with protein side chains are indicated by lines connecting to the respective residue box, while interactions to main chain atoms are shown as lines to the specific main chain atoms indicated. van der Waals contacts are indicated by dotted lines and hydrogen bonds by thick broken lines; (**C**). Schematic representation of the ATP binding pocket of CDK2; (**D**) Schematic representation of the ATP binding pocket of CDK2 in the complex with des-chloroflavopiridol. Source of A and B: [111]; C and D: [112].

The adenine ring is enclosed in a hydrophobic pocket formed by Ile^{10}, Ala^{31}, Val^{64}, Phe^{80}, Phe^{82}, and Leu^{134} (Fig. **9A** and **9B**) and forms two hydrogen bonds, between the N6 atom of the adenine and the carbonyl oxygen of Glu^{81}, and between N1 and the backbone amide of Leu^{83}. Key hydrogen bonds are formed to Lys^{33} and Wat^{384} with O(3) of the hydroxyl-piperidine moiety and to Asp^{145} with the N(11)H and O(3)H. Core hydrogen bonds are formed to Glu^{81} with O(5)H and to Leu^{83} with O(4).

As shown in Fig. (**9C**-**9D**), compound **53** essentially takes the same position as ATP, with the flavonoid ring system and the adenine lying in identical planes, but twisted approximately 60° to each other. CDK2 binding is mainly achieved by hydrophobic interactions. Of special interest is the phenyl group of inhibitor, since it points out of the ATP binding pocket of the enzymes and occupies an area that cannot be utilized by ATP. This additional interaction seems to be responsible for the high affinity and selectivity of inhibitor for CDK1 and CDK2 in comparison to other protein kinases, such as PKA. The chloro substituent (compound **11**) leads to a tenfold increase in activity. Its higher affinity is caused by an improved interaction with an isoleucine side chain [44].

Based on computational studies, structure activity relationship has been deduced. Chromone moiety of the inhibitor is essential for inhibitory activity, it acts as a mimetic of the purine moiety of ATP, the 4-keto and 5-hydroxy groups are essential for bidendate hydrogen bonds with the backbone of CDK2 residues Leu 83 and Glu 81 [32], methyl ether was found to be inactive. Replacement of chromenone with coumarin, isocoumarin and quinolone, resulted in reduced activity [48]. Schoepfer *et al*. have shown that the 5-hydroxychromone skeleton of flavopiridol can be replaced by a 4- hydroxybenzofuranone [45]. The presence of the nitrogen in ring D is critical since it provide hydrophilic interaction with Asp145 and introduction of second heteroatom (for example hydroxyl group) in ring increases inhibitory potency [112]. Phenyl group (ring C) is important. Hydrophobic side chains such as chlorophenyl or *tert*-butyl produced potent inhibitory activity, since it points out of the ATP binding pocket of the enzymes and occupies an area that cannot be utilized by ATP. This additional interaction seems to be responsible for the high affinity and selectivity of inhibitor for CDK-1 and CDK-2. The flavopiridol leads to a ten-fold increase in activity than des-chloroflavopiridol because an improved interaction with an isoleucine side chain of enzyme [35, 112]. Other substitutions are tolerable at phenyl ring; for example, 4-nitro derivative of P276-00 (**12**) is potent CDK inhibitor [113]. Hydrophilic side chains such as pyrimidine or aniline caused a severe reduction in CDK inhibitory activity [44]. The introduction of a sulfur or an oxygen linker increases the selectivity towards CDK-1/B over CDK-2/E and CDK-4/D1 [44]. The SAR of flavopiridol is summarized in Fig. (**10**).

CONCLUSIONS

Over 60% of the current anticancer drugs have their origin in one way or another from natural sources. Amongst these, flavonoids have a great potential to emerge as therapeutic agents. Naturally occurring flavone alkaloid rohitukine (**10**) has already delivered two clinical candidates flavopiridol (**11**) and P-276-00 (**12**). These compounds competitively bind to the ATP binding pocket of CDKs, although they have shown a broad inhibitory capacity of several CDKs. First amongst these, flavopiridol (**11**) has already been approved as a orphan drug for treatment of chronic lymphocytic leukaemia whereas, later molecule P-276-00 (**12**) is currently in phase II clinical studies for advanced refractory neoplasms and multiple myeloma. Flavopiridol as a single agent has shown disappointing results for treatment of solid tumors due to PK related problems. Further medicinal chemistry efforts may come up with candidates with improved PK parameters. Thus, there exists a great potential in flavone alkaloid class of compounds to come up with more clinical candidates for anticancer therapy. In addition, several compounds were also potent inhibitors of positive transcription elongation factor b (P-TEFb, CDK-9; for example, **69**: 6.5 nM; **67**: 9 nM) which plays important role in HIV replication; thus these compounds can be potential leads for anti-HIV drug development program.

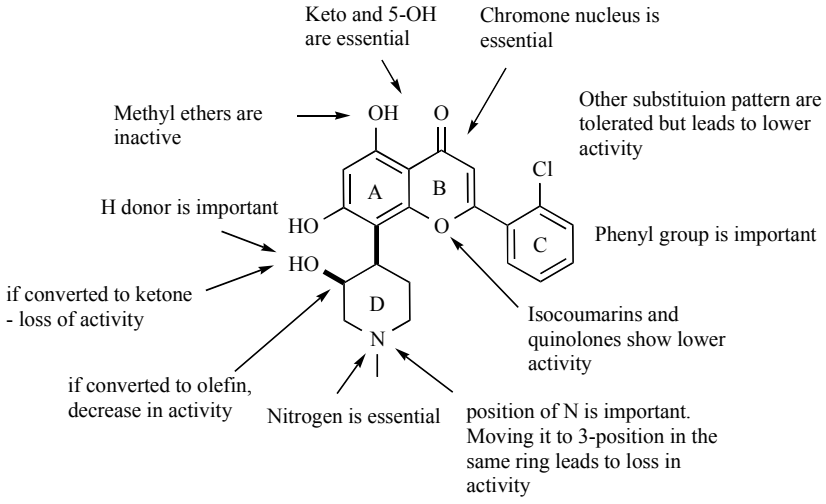


Fig. (10). SAR of flavopiridol (**11**).

REFERENCES

[1] Martens, S.; Mithofer, A. Flavones and flavone synthases. *Phytochemistry* **2005**, *66*, 2399-407.

[2] Mizutani, M.; Tanaka, Y.; Kusumi, T.; Ayabe, S.; Akashi, T. Flavone synthases, methods of using flavone synthases, and plants expressing flavone synthases. *U.S Patent 7465569 (International Flower Developments Proprietary Limited, Australia)* **2008**.

[3] Pichersky, E.; Gang, D.R. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plant Sci.* **2000**, *5*, 439-45.

[4] Spaink, H.P. The molecular basis of infection and nodulation by *Rhizobia*: The ins and outs of sympathogenesis. *Annu. Rev. Phytopathol.* **1995**, *33*, 345–68.

[5] Harborne, J.B.; Williams, C.A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481-504.

[6] Pietta, P.; Minoggio, M.; Bramati, L.; Rahman, A.-U. Plant polyphenols: Structure, occurrence and bioactivity. In *Studies in Natural Products Chemistry*, Elsevier: **2003**; Vol. 28, Part 9, pp 257-312.

[7] Verma, A.K.; Pratap, R. The biological potential of flavones. *Nat. Prod. Rep.* **2010**, *27*, 1571-93.

[8] Kosmider, B.; Osiecka, R. Flavonoid compounds: A review of anticancer properties and interactions with cis-Diamminedichloroplatinum(II). *Drug Dev. Res.* **2004**, *63*, 200-11.

[9] Ren, W.; Qiao, Z.; Wang, H.; Lei Zhu, L.Z. Flavonoids: Promising anticancer agents. *Med. Res. Rev.* **2003**, *23*, 519-34.

[10] Teillet, F.; Boumendjel, A.; Boutonnat, J.; Ronot, X. Flavonoids as RTK inhibitors and potential anticancer agents. *Med. Res. Rev.* **2008**, *28*, 715-45.

[11] Wang, H.K. The therapeutic potential of flavonoids. *Expert Opin. Investig. Drugs* **2000**, *9*, 2103-19.

[12] Wang, H.K.; Xia, Y.; Yang, Z.Y.; Natschke, S.L.; Lee, K.H. Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. *Adv. Exp. Med. Biol.* **1998**, *439*, 191–225.

[13] Boumendjel, A.; Pietro, A.D.; Dumontet, C.; Barron, D. Recent advances in the discovery of flavonoids and analogs with high-affinity binding to P-glycoprotein responsible for cancer cell multidrug resistance. *Med. Res. Rev.* **2002**, *22*, 512-29.

[14] Roger, C.R. The nutritional incidence of flavonoids: Some physiological and metabolic considerations. *Experientia* **1988**, *44*, 725–33.

[15] Cotelle, N.; Bernier, J.L.; Henichart, J.P.; Catteau, J.P.; Gaydou, E.; Wallet, J.C. Scavenger and antioxidant properties of ten synthetic flavones. *Free Radic. Biol. Med.* **1992**, *13*, 211–9.

[16] DiCarlo, G.; Mascolo, N.; Izzo, A.A.; Capasso, F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* **1999**, *65*, 337–53.

[17] Kuntz, S.; Wenzel, U.; Daniel, H. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. *Eur. J. Nutr.* **1999**, *38*, 133–42.

[18] Choi, S.U.; Ryu, S.Y.; Yoon, S.K.; Jung, N.P.; Park, S.H.; Kim, K.H.; Choi, E.J.; Lee, C.O. Effects of flavonoids on the growth and cell cycle of cancer cells. *Anticancer Res.* **1999**, *19*, 5229–33.

[19] Kaulich, M.; Streicher, F.; Mayer, R.; Muller, I.; Muller, C.E. Flavonoids - Novel lead compounds for the development of P2Y2 receptor antagonists. *Drug Dev. Res.* **2003**, *59*, 72–81.

[20] Campbell, D.R.; Kurzer, M.S. Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. *J. Steroid Biochem. Mol. Biol.* **1993**, *46*, 381-8.

[21] Ibrahim, A.R.; Abul-Hajj, Y.J. Aromatase inhibition by flavonoids. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 257-60.

[22] Yamashita, Y.; Kawada, S.; Nakano, H. Induction of mammalian topoisomerase II dependent DNA cleavage by nonintercalative flavonoids, genistein and orobol. *Biochem. Pharmacol.* **1990**, *39*, 737–44.

[23] Ferriola, P.C.; Cody, V.; Middleton, E. Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem. Pharmacol.* **1989**, *38*, 1617-24.

[24] Lin, C.W.; Hou, W.C.; Shen, S.C.; Juan, S.H.; Ko, C.H.; Wang, L.M.; Chen, Y.C. Quercetin inhibition of tumor invasion via suppressing PKC delta/ERK/AP-1-dependent matrix metalloproteinase-9 activation in breast carcinoma cells. *Carcinogenesis* **2008**, *29*, 1807-15.

[25] Choi, J.A.; Kim, J.Y.; Lee, J.Y.; Kang, C.M.; Kwon, H.J.; Yoo, Y.D.; Kim, T.W.; Lee, Y.S.; Lee, S.J. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int. J. Oncol.* **2001**, *19*, 837-44.

[26] Zi, X.; Feyes, D.K.; Agarwal, R. Anticarcinogenic effect of a flavonoid antioxidant, silymarin, in human breast cancer cells MDA-MB 468: Induction of G1 arrest through an Increase in Cip1/p21 concomitant with a decrease in kinase activity of cyclin-dependent kinases and associated cyclins. *Clin. Cancer Res.* **1998**, *4*, 1055–64.

[27] Cushman, M.; Zhu, H.; Geahlen, R.L.; Kraker, A.J. Synthesis and biochemical evaluation of a series of aminoflavones as potential inhibitors of protein-tyrosine kinases p56lck, EGFr, and p60v-src. *J. Med. Chem.* **1994**, *37*, 3353-62.

[28] Senderowicz, A.M. Development of cyclin-dependent kinase modulators as novel therapeutic approaches for hematological malignancies. *Leukemia* **2001**, *15*, 1-9.

[29] Neant, I.; Guerrier, P. 6-Dimethylaminopurine blocks starsh oocyte maturation by inhibiting a relevant protein kinase activity. *Exp. Cell Res.* **1988**, *176*, 68-79.

[30] Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J.J.; Donella-Deana, A.; Pinna, L.; Letham, D.S.; Kato, J.Y.; DeÂtivaud, L.; Leclerc, J.; Meijer, L. Inhibition of cyclin-dependent kinases by purine derivatives. *Eur. J. Biochem.* **1994**, *224*, 771-9.

[31] Meijer, L.; Borgne, A.; Mulner, O.; Chong, J.P.; Blow, J.J.; Inagaki, N.; Inagaki, M.; Delcros, J.G.; Moulinoux, J.P. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur. J. Biochem.* **1997**, *243*, 527-36.

[32] Gray, N.; Detivaud, L.; Doerig, C.; Meijer, L. ATP-site directed inhibitors of cyclin-dependent kinases. *Curr. Med. Chem.* **1999**, *6*, 859-75.

[33] Gray, N.S.; Wodicka, L.; Thunnissen, A.M.; Norman, T.C.; Kwon, S.; Espinoza, F.H.; Morgan, D.O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.H.; Lockhart, D.J.; Schultz, P.G. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **1998**, *281*, 533-8.

[34] Lu, H.; Chang, D.J.; Baratte, B.; Meijer, L.; Schulze-Gahmen, U. Crystal structure of a human cyclin-dependent kinase 6 complex with a flavonol inhibitor fisetin. *J. Med. Chem.* **2005**, *48*, 737-43.

[35] Zhang, S.; Ma, J.; Bao, Y.; Yang, P.; Zou, L.; Li, K.; Sun, X. Nitrogen-containing flavonoid analogues as CDK1/cyclin B inhibitors: synthesis, SAR analysis, and biological activity. *Bioorg. Med. Chem.* **2008**, *16*, 7128-33.

[36] Lee, H.Z.; Leung, H.W.; Lai, M.Y.; Wu, C.H. Baicalein induced cell cycle arrest and apoptosis in human lung squamous carcinoma CH27 cells. *Anticancer Res.* **2005**, *25*, 959-64.

[37] Vijayababu, M.R.; Kanagaraj, P.; Arunkumar, A.; Ilangovan, R.; Aruldhas, M.M.; Arunakaran, J. Quercetin-induced growth inhibition and cell death in prostatic carcinoma cells (PC-3) are associated with increase in p21 and hypophosphorylated retinoblastoma proteins expression. *J. Cancer Res. Clin. Oncol.* **2005**, *131*, 765-71.

[38] Khadem, S.; Marles, R.J. Chromone and flavonoid alkaloids: Occurrence and bioactivity. *Molecules* **2012**, *17*, 191-206.

[39] Harmon, A.D.; Weiss, U.; Silverton, J.V. The structure of rohitukine, the main alkaloid of *Amoora rohituka* (syn. *Aphanamixis polystachya*) (Meliaceae). *Tetrahedron Lett.* **1979**, *8*, 721-4.

[40] Naik, R.G.; Kattige, S.L.; Bhat, S.V.; Alreja, B.; de Souza, N.J.; Rupp, R.H. An antiinflammatory cum immunomodulatory piperidinylbenzopyranone from *Dysoxylum binectariferum*: isolation, structure and total synthesis. *Tetrahedron* **1988**, *44*, 2081-6.

[41] Wang, L.M.; Ren, D.M. Flavopiridol, the first cyclin-dependent kinase inhibitor: recent advances in combination chemotherapy. *Mini-Rev. Med. Chem.* **2010**, *10*, 1058-70.

[42] Kattige, S.L.; Naik, R.G.; Lakdawalla, A.D.; Alihussein, N.D.; Rupp, R.H.; Souza, N.J. 4H-Benzopyran-4-one Compounds Which Have Antiinflammatory or Immunomodulating Action. U.S. Patent 4,900,727, Feb 13, 1990.

[43] Tabaka, A.C.; Murthi, K.K.; Pal, K.; Teleha, C.A. Application of modified flavone closure for the preparation of racemic L86-8275. *Org. Proc. Res. Dev.* **1999**, *3*, 256-9.

[44] Kim, K.S.; Sack, J.S.; Tokarski, J.S.; Qian, L.; Chao, S.T.; Leith, L.; Kelly, Y.F.; Misra, R.N.; Hunt, J.T.; Kimball, S.D.; Humphreys, W.G.; Wautlet, B.S.; Mulheron, J.G.; Webster, K.R. Thio- and oxoflavopiridols, cyclin-dependent kinase 1-selective inhibitors: synthesis and biological effects. *J. Med. Chem.* **2000**, *43*, 4126-34.

[45] Schoepfer, J.; Fretz, H.; Chaudhuri, B.; Muller, L.; Seeber, E.; Meijer, L.; Lozach, O.; Vangrevelinghe, E.; Furet, P. Structure-based design and synthesis of 2-benzylidene-benzofuran-3-ones as flavopiridol mimics. *J. Med. Chem.* **2002**, *45*, 1741-7.

[46] Sivakumar, M.; Shukla, M.; Jadhav, P.K.; Borhade, A. An enantioselective synthesis of pyrrolidine-substituted flavones. WO2008007169 (Piramal Life Science Ltd. India), 2008.

[47] Ali, A.; Ghosh, A.; Nathans, R.S.; Sharova, N.; O'Brien, S.; Cao, H.; Stevenson, M.; Rana, T.M. Identification of flavopiridol analogues that selectively inhibit positive transcription elongation factor (P-TEFb) and block HIV-1 replication. *ChemBioChem* **2009**, *10*, 2072-80.

[48] Murthi, K.K.; Dubay, M.; McClure, C.; Brizuela, L.; Boisclair, M.D.; Worland, P.J.; Mansuri, M.M.; Pal, K. Structure-activity relationship studies of flavopiridol analogues. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1037-41.

[49] Ahn, Y.M.; Vogeti, L.; Liu, C.J.; Santhapuram, H.K.; White, J.M.; Vasandani, V.; Mitscher, L.A.; Lushington, G.H.; Hanson, P.R.; Powell, D.R.; Himes, R.H.; Roby, K.F.; Ye, Q.; Georg, G.I. Design, synthesis, and antiproliferative and CDK2-cyclin a inhibitory activity of novel flavopiridol analogues. *Bioorg. Med. Chem.* **2007**, *15*, 702-13.

[50] Joshi, K.S.; Rathos, M.J.; Joshi, R.D.; Sivakumar, M.; Mascarenhas, M.; Kamble, S.; Lal, B.; Sharma, S. *In vitro* antitumor properties of a novel cyclin-dependent kinase inhibitor P276-00. *Mol. Cancer Ther.* **2007**, *6*, 918-25.

[51] Raje, N.; Hideshima, T.; Mukherjee, S.; Raab, M.; Vallet, S.; Chhetri, S.; Cirstea, D.; Pozzi, S.; Mitsiades, C.; Rooney, M.; Kiziltepe, T.; Podar, K.; Okawa, Y.; Ikeda, H.; Carrasco, R.; Richardson, P.G.; Chauhan, D.; Munshi, N.C.; Sharma, S.; Parikh, H.; Chabner, B.; Scadden, D.; Anderson, K.C. Preclinical activity of P276-00, a novel small-molecule cyclin-dependent kinase inhibitor in the therapy of multiple myeloma. *Leukemia* **2009**, *23*, 961-70.

[52] Senderowicz, A.M. Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials. *Invest. New Drugs* **1999**, *17*, 313-20.

[53] Kaur, G.; Stetler-Stevenson, M.; Sebers, S.; Worland, P.; Sedlacek, H.; Myers, C.; Czech, J.; Naik, R.; Sausville, E. Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. *J. Natl. Cancer Inst.* **1992**, *84*, 1736-40.

[54] Losiewicz, M.D.; Carlson, B.A.; Kaur, G.; Sausville, E.A.; Worland, P.J. Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 589-95.

[55] Sedlacek, H.H. Mechanisms of action of flavopiridol. *Crit. Rev. Oncol. Hematol.* **2001**, *38*, 139-70.

[56] Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J.A.; Snyder, G.L.; Greengard, P.; Biernat, J.; Wu, Y.Z.; Mandelkow, E.M.; Eisenbrand, G.; Meijer, L. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* **2001**, *276*, 251-60.

[57] Carlson, B.A.; Dubay, M.M.; Sausville, E.A.; Brizuela, L.; Worland, P.J. Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res.* **1996**, *56*, 2973-8.

[58] Kelland, L.R. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. *Expert Opin. Investig. Drugs* **2000**, *9*, 2903-11.

[59] Worland, P.J.; Kaur, G.; Stetler-Stevenson, M.; Sebers, S.; Sartor, O.; Sausville, E.A. Alteration of the phosphorylation state of p34cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. Correlation with decreased H1 kinase activity. *Biochem. Pharmacol.* **1993**, *46*, 1831-40.

[60] Kim, D.M.; Koo, S.Y.; Jeon, K.; Kim, M.H.; Lee, J.; Hong, C.Y.; Jeong, S. Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-a or TNF-related apoptosis-inducing ligand in human cancer cell lines. *Cancer Res.* **2003**, *63*, 621–6.

[61] Cartee, L.; Maggio, S.C.; Smith, R.; Sankala, H.M.; Dent, P.; Gran, S. Protein kinase C-dependent activation of the Tumor necrosis factor receptor-mediated extrinsic cell Death pathway underlies enhanced apoptosis in human myeloid leukemia cells exposed to bryostatin 1 and flavopiridol. *Mol. Cancer Ther.* **2003**, *2*, 83-93.

[62] Wu, K.; Wang, C.; D'Amico, M.; Lee, R.J.; Albanese, C.; Pestell, R.G.; Mani, S. Flavopiridol and trastuzumab synergistically inhibit proliferation of breast cancer cells: Association with selective cooperative inhibition of cyclin D1-dependent kinase and Akt signaling pathways. *Mol. Cancer Ther.* **2002**, *1*, 695-706.

[63] Nahta, R.; Iglehart, J.D.; Kempkes, B.; Schmidt, E.V. Rate-limiting effects of cyclin D1 in transformation by ErbB2 predicts synergy between herceptin and flavopiridol. *Cancer Res.* **2002**, *62*, 2267-71.

[64] Arguello, F.; Alexander, M.; Sterry, J.A.; Tudor, G.; Smith, E.M.; Kalavar, N.T.; Greene, J.F.; Koss, W.; Morgan, C.D.; Stinson, S.F.; Siford, T.J.; Alvord, W.G.; Klabansky, R.L.; Sausville, E.A. Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity *In vivo* against human leukemia and lymphoma xenografts. *Blood* **1998**, *91*, 2482-90.

[65] Gojo, I.; Zhang, B.; Fenton, R.G. The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down regulation of Mcl-1. *Clin. Cancer Res.* **2002**, *8*, 3527–38.

[66] Wittmann, S.; Bali, P.; Donapaty, S.; Nimmanapalli, R.; Guo, F.; Yamaguchi, H.; Huang, M.; Jove, R.; Wang, H.G.; Bhalla, K. Flavopiridol down-regulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis. *Cancer Res.* **2003**, *63*, 93-9.

[67] Carlson, B.; Lahusen, T.; Singh, S.; Loaiza-Perez, A.; Worland, P.J.; Pestell, R.; Chris Albanese; Sausville, E.A.; Senderowicz, A.M. Down-regulation of cyclin D1 by transcriptional repression in MCF-7 human breast carcinoma cells induced by flavopiridol. *Cancer Res.* **1999**, *59*, 4634-41.

[68] Rapella, A.; Negrioli, A.; Melillo, G.; Pastorino, S.; Varesio, L.; Bosco, M.C. Flavopiridol inhibits vascular endothelial growth factor production induced by hypoxia or picolinic acid in human neuroblastoma. *Int. J. Cancer* **2002**, *99*, 658–64.

[69] Melillo, G.; Sausville, E.A.; Cloud, K.; Lahusen, T.; Varesio, L.; Senderowicz, A.M. Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. *Cancer Res.* **1999**, *59*, 5433–7.

[70] Takada, Y.; Aggarwal, B.B. Flavopiridol inhibits NF-kB activation induced by various carcinogens and inflammatory agents through inhibition of IkBa kinase and p65 phosphorylation. *J. Biol. Chem.* **2004**, *279*, 4750–9.

[71] Michalides, R.; van Veelen, N.; Hart, A.; Loftus, B.; Wientjens, E.; Balm, A. Overexpression of cyclin D1 correlates with recurrence in a group of forty-seven operable squamous cell carcinomas of the head and neck. *Cancer Res.* **1995**, *55*, 975-8.

[72] Brusselbach, S.; Nettelbeck, D.M.; Sedlacek, H.H.; Muller, R. Cell cycle-independent induction of apoptosis by the anti-tumor drug Flavopiridol in endothelial cells. *Int. J. Cancer* **1998**, *77*, 146-52.

[73] Kerr, J.S.; Wexler, R.S.; Mousa, S.A.; Robinson, C.S.; Wexler, E.J.; Mohamed, S.; Voss, M.E.; Devenny, J.J.; Czerniak, P.M.; Gudzelak, A.; Slee, A.M. Novel small molecule alpha v integrin antagonists: comparative anti-cancer efficacy with known angiogenesis inhibitors. *Anticancer Res.* **1999**, *19*, 959-68.

[74] Sedlacek, H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. Flavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy. *Int. J. Oncol.* **1996**, *9*, 1143-68.

[75] Kelland, L.R. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. *Expert Opin. Investig. Drugs* **2000**, *9*, 2903-11.

[76] Drees, M.; Dengler, W.A.; Roth, T.; Labonte, H.; Mayo, J.; Malspeis, L.; Grever, M.; Sausville, E.A.; Fiebig, H.H. Flavopiridol (L86-8275): selective antitumor activity *in vitro* and activity *in vivo* for prostate carcinoma cells. *Clin. Cancer Res.* **1997**, *3*, 273-9.

[77] Byrd, J.C.; Shinn, C.; Waselenko, J.K.; Fuchs, E.J.; Lehman, T.A.; Nguyen, P.L. Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without

evidence of bcl-2 modulation or dependence on functional p53. *Blood* **1998**, *92*, 3804–16.

[78] Thomas, J.P.; Tutsch, K.D.; Cleary, J.F.; Bailey, H.H.; Arzoomanian, R.; Alberti, D. Phase I clinical and pharmacokinetic trial of the cyclin-dependent kinase inhibitor flavopiridol. *Cancer Chemother. Pharmacol.* **2002**, *50*, 465–72.

[79] Parker, B.W.; Kaur, G.; Nieves-Neira, W.; Taimi, M.; Kohlhagen, G.; Shimizu, T.; Losiewicz, M.D.; Pommier, Y.; Sausville, E.A.; Senderowicz, A.M. Early induction of apoptosis in hematopoietic cell lines after exposure to flavopiridol. *Blood* **1998**, *91*, 458-65.

[80] Arguello, F.; Alexander, M.; Sterry, J.A.; Tudor, G.; Smith, E.M.; Kalavar, N.T.; Greene, J.F., Jr.; Koss, W.; Morgan, C.D.; Stinson, S.F.; Siford, T.J.; Alvord, W.G.; Klabansky, R.L.; Sausville, E.A. Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity *In vivo* against human leukemia and lymphoma xenografts. *Blood* **1998**, *91*, 2482-90.

[81] Bradbury, R.H. Kinase inhibitors: Approved drugs and clinical candidates. In *Burger's medicinal chemistry, drug discovery, and development*, 7th ed.; Abraham, D.J.; Rotella, D.P., Eds. John Wiley & Sons, Inc.: **2010**.

[82] Diaz-Padilla, I.; Siu, L.L.; Duran, I. Cyclin-dependent kinase inhibitors as potential targeted anticancer agents. *Invest. New Drugs* **2009**, *27*, 586–94.

[83] Burdette-Radoux, S.; Tozer, R.G.; Lohmann, R.C.; Quirt, I.; Ernst, D.S.; Walsh, W. Phase II trial of flavopiridol, a cyclin dependent kinase inhibitor in untreated metastatic malignant melanoma. *Invest. New Drugs* **2004**, *22*, 315–22.

[84] Liu, G.; Gandara, D.R.; Lara, P.; Raghavan, D.; Doroshow, J.H.; Twardowski, P. A Phase II trial of flavopiridol (NSC #649890) in patients with previously untreated metastatic androgen-independent prostate cancer. *Clin. Cancer Res.* **2004**, *10*, 924–8.

[85] Stadler, W.M.; Vogelzang, N.J.; Amato, R.; Sosman, J.; Taber, D.; Liebowitz, D.; Vokes, E.E. Flavopiridol, a novel cyclin-dependent kinase inhibitor in metastatic renal cancer: A University of Chicago Phase II consortium study. *J. Clin. Oncol.* **2000**, *18*, 371-5.

[86] Brown, J.R. Chronic lymphocytic leukemia: a niche for flavopiridol? *Clin. Cancer Res.* **2005**, *11*, 3971–3.

[87] Christian, B.A.; Grever, M.R.; Byrd, J.C.; Lin, T.S. Flavopiridol in the treatment of chronic lymphocytic leukemia. *Curr. Opin. Oncol.* **2007**, *19*, 573–8.

[88] Misra, R.N. Clinical progress of selective cyclin dependent kinase (CDK) inhibitors. *Drugs Future* **2006**, *31*, 43–52.

[89] Blagosklonny, M.V. Flavopiridol, an inhibitor of transcription. Implications, problems and solutions. *Cell Cycle* **2004**, *3*, 1537-42.

[90] Getman, C.R.; Bible, K.C. Molecular targets of flavopiridol, a promising new antineoplastic agent. *Recent Res. Dev. Cancer* **2004**, *6*, 37-56.

[91] Phelps, M.A.; Lin, T.S.; Johnson, A.J.; Hurh, E.; Rozewski, D.M.; Farley, K.L.; Wu, D.; Blum, K.A.; Fischer, B.; Mitchell, S.M.; Moran, M.E.; Brooker-McEldowney, M.; Heerema, N.A.; Jarjoura, D.; Schaaf, L.J.; Byrd, J.C.; Grever, M.R.; Dalton, J.T. Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. *Blood* **2009**, *113*, 2637-45.

[92] Dispenzieri, A.; Gertz, M.A.; Lacy, M.Q.; Geyer, S.M.; Fitch, T.R.; Fenton, R.G.; Fonseca, R.; Isham, C.R.; Ziesmer, S.C.; Erlichman, C.; Bible, K.C. Flavopiridol in patients with relapsed or refractory multiple myeloma: a phase 2 trial with clinical and pharmacodynamic end-points. *Haematologica* **2006**, *91*, 390-3.

[93] Byrd, J.C.; Lin, T.S.; Dalton, J.T.; Wu, D.; Phelps, M.A.; Fischer, B.; Moran, M.; Blum, K.A.; Rovin, B.; Brooker-McEldowney, M.; Broering, S.; Schaaf, L.J.; Johnson, A.J.; Lucas, D.M.; Heerema, N.A.; Lozanski, G.; Young, D.C.; Suarez, J.-R.; Colevas, A.D.; Grever, M.R. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. *Blood* **2007**, *109*, 399-404.

[94] Motwani, M.; Jung, C.; Sirotnak, F.M.; She, Y.; Shah, M.A.; Gonen, M.; Schwartz, G.K. Augmentation of apoptosis and tumor regression by flavopiridol in the presence of CPT-11 in Hct116 colon cancer monolayers and xenografts. *Clin. Cancer Res.* **2001**, *7*, 4209-19.

[95] Shah, M.A.; Schwartz, G.K. Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. *Clin. Cancer Res.* **2001**, *7*, 2168-81.

[96] Bible, K.C.; Kaufmann, S.H. Cytotoxic synergy between flavopiridol (NSC 649890, L86-8275) and various antineoplastic agents: The importance of sequence of administration. *Cancer Res.* **1997**, *57*, 3375-80.

[97] Cartee, L.; Wang, Z.; Decker, R.H.; Chellappan, S.P.; Fusaro, G.; Hirsch, K.G.; Sankala, H.M.; Dent, P.; Grant, S. The cyclin-dependent kinase inhibitor (CDKI) flavopiridol disrupts phorbol 12-myristate 13-acetate-induced differentiation and CDKI expression while enhancing apoptosis in human myeloid leukemia cells. *Cancer Res.* **2001**, *61*, 2583–91.

[98] Motwani, M.; Rizzo, C.; Sirotnak, F.; She, Y.; Schwartz, G.K. Flavopiridol enhances the effects of docetaxel *in vitro* and *in vivo* in human gastric cancer cells. *Mol. Cancer Ther.* **2003**, *2*, 549–55.

[99] Motwani, M.; Delohery, T.M.; Schwartz, G.K. Sequential dependent enhancement of caspase activation and apoptosis by flavopiridol on paclitaxel-treated human gastric and breast cancer cells. *Clin. Cancer Res.* **1999**, *5*, 1876–83.

[100] El-Rayes, B.F.; Gadgeel, S.; Parchment, R.; Lorusso, P.; Philip, P.A. A phase I study of flavopiridol and docetaxel. *Invest. New Drugs* **2006**, *24*, 305–10.

[101] Shah, M.A.; Kortmansky, J.; Motwani, M.; Drobnjak, M.; Gonen, M.; Yi, S. A phase I clinical trial of the sequential combination of irinotecan followed by flavopiridol. *Clin. Cancer Res.* **2005**, *11*, 3836-45.

[102] Fornier, M.N.; Rathkopf, D.; Shah, M.A.; Patil, S.; O'Reilly, E.; Tse, A.N. Phase I dose-finding study of weekly docetaxel followed by flavopiridol for patients with advanced solid tumors. *Clin. Cancer Res.* **2007**, *13*, 5841-6.

[103] Carvajal, R.D.; Shah, M.A.; Tse, R. A phase II study of docetaxel followed by flavopiridol in advanced, gemcitabine-refractory pancreatic cancer. *Proc. Am. Soc. Clin Oncol. abstr.* **2008**, 15558.

[104] George, S.; Kasimis, B.S.; Cogswell, J.; Schwarzenberger, P.; Shapiro, G.I.; Fidias, P. Phase I study of flavopiridol in combination with Paclitaxel and Carboplatin in patients with non-small-cell lung cancer. *Clin. Lung Cancer* **2008**, *9*, 160–5.

[105] Dickson, M.A.; Shah, M.A.; Rathkopf, D.; Tse, A.; Carvajal, R.D.; Wu, N.; Lefkowitz, R.A.; Gonen, M.; Cane, L.M.; Dials, H.J.; Schwartz, G.K. A phase I clinical trial of FOLFIRI in combination with the pan-cyclin-dependent kinase (CDK) inhibitor flavopiridol. *Cancer Chemother. Pharmacol.* **2010**, *66*, 1113–21.

[106] Holkova, B.; Perkins, E.B.; Ramakrishnan, V.; Tombes, M.B.; Shrader, E.; Talreja, N.; Wellons, M.D.; Hogan, K.T.; Roodman, G.D.; Coppola, D.; Kang, L.; Dawson, J.; Stuart, R.K.; Peer, C.; Figg, W.D.; Kolla, S.; Doyle, A.; Wright, J.; Sullivan, D.M.; Roberts, J.D.; Grant, S. Phase I Trial of Bortezomib (PS-341; NSC 681239) and Alvocidib (Flavopiridol; NSC 649890) in patients with recurrent or refractory B-cell neoplasms. *Clin. Cancer Res.* **2011**, *17*, 3388-97.

[107] Hirte, H.W.; Raghunadharao, D.; Baetz, T.; Hotte, S.; Rajappa, S.; Iacobucci, A.; Sharma, S.; Parikh, H.; Kulkarni, S.; Patil, S.; Gaston, S. A phase 1 study of the selective cyclin dependent kinase inhibitor P276-00 in patients with advanced refractory neoplasms. *J. Clin. Oncol. (Abstract: ASCO Annual Meeting Proceedings)* **2007**, *25*, 14117.

[108] Joshi, K.S.; Rathos, M.J.; Mahajan, P.; Wagh, V.; Shenoy, S.; Bhatia, D.; Chile, S.; Sivakumar, M.; Maier, A.; Fiebig, H.H.; Sharma, S. P276-00, a novel cyclin-dependent inhibitor induces G1-G2 arrest, shows antitumor activity on cisplatin-resistant cells and significant *in vivo* efficacy in tumor models. *Mol. Cancer Ther.* **2007**, *6*, 926-34.

[109] Giridharan, P.; Chakrabarti, D.; Khanna, A.; Ved, U.; Almeida, A.; Sharma, S.; Padigaru, M.; Balakrishnan, A. Novel syngergistic combination of gemcitabine with P-276-00 or PI446A in treatment of cancer. US2010/0152129 (Piramal Life Science Ltd. India), 2010.

[110] Rathos, M.; Joshi, K.; Khanwalkar, H.; Sharma, S. A synergistic pharmaceutical combination for the treatment of cancer. WO2008/139271 (Piramal Life Sciences Ltd. India), 2008.

[111] De Azevedo, W.F., Jr.; Mueller-Dieckmann, H.J.; Schulze-Gahmen, U.; Worland, P.J.; Sausville, E.; Kim, S.H. Structural basis for specificity and potency of a flavonoid inhibitor of human

CDK2, a cell cycle kinase. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2735-40.

[112] Huwe, A.; Mazitschek, R.; Giannis, A. Small molecules as inhibitors of cyclin-dependent kinases. *Angew. Chem. Int. Ed.* **2003**, *42*, 2122-38.

[113] Galons, H.; Oumata, N.; Meijer, L. Cyclin-dependent kinase inhibitors: a survey of recent patent literature. *Expert Opin. Ther. Patents* **2010**, *20*, 377-404.

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